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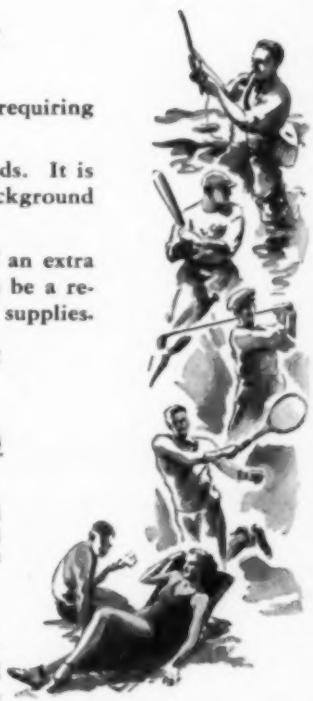

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*Scientific Edition*

# JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

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## Local Anesthetic Action of Some Aminonaphthoic Acid Esters

*By L. W. Rowe**

For a number of years pharmacological work has been in progress in our laboratory on a series of esters of aminonaphthoic acids. These compounds have been prepared and the chemical data reported by Blicke and Parke (1). Very little can be found in the literature about the action of derivatives of naphthoic acid. Fisk and Underhill (2) have reported on the anesthetic action of esters of $\alpha$- and $\beta$-naphthoic acid and also certain alkoxynaphthoic acids prepared by Hill and co-workers. In addition, when work in this laboratory already had been in progress for some time, two Russian chemists (3) described four esters of 4-amino-1-naphthoic acid and reported them to have marked local anesthetic action.

The naphthyl analog of procaine, namely, the diethylaminoethyl ester of 4-amino-1-naphthoic acid, as the hydrochloride ($C_{17}H_{22}O_2N_2.HCl$) was the first to be investigated in this laboratory and proved to have several desirable properties. It will be designated hereafter as Naphthocaine 4*A*.

* From the Research Laboratories of Parke, Davis & Co., Detroit, Mich.

### EXPERIMENTAL

The two methods used chiefly for determining local anesthetic efficiency were the rabbit cornea and the frog sensory nerve as proposed by Sollmann (4) but the human wheal method was used in a few instances (5). Toxicity was determined by intraperitoneal injection into white mice and usually only three animals were used for the critical dose since great accuracy involving the use of large groups was not essential. Many other comparative effects with cocaine and procaine were determined on various animals and by a large number of methods.

In Table I are given the chemical names and formulas of the series of compounds investigated in comparison with cocaine and procaine. These are called naphthocaines and the symbol is given by which they are designated throughout this article.

The monohydrochlorides of these esters were all soluble in distilled water to at least 0.5% but it was found that the sulfamate ($NH_2.SO_3H$) salts were more soluble, so a few compounds were prepared and tested in that form also.

In Table II is given the comparative toxicity of these compounds when injected into white mice intrapertioneally as well as miscellaneous effects such as local irritant action, blood pressure action, etc. Large groups of mice were not used at the critical dose but the M. L. D. killed 3 out of 5 or at least 2 out of 3.

### DISCUSSION

About half of these compounds show no appreciable local irritant action in concen-

Table I.—Names and Symbols of Naphthocaines

| Chemical Name | Formula as HCl | Symbol |
|---|---|---|
| **Esters of 3-amino-1-naphthoic acid** | | |
| 1. Beta-diethylaminoethyl | $C_{17}H_{22}O_2N_2.HCl$ | 3*A* |
| 2. Beta di-*n*-butylaminoethyl | $C_{21}H_{30}O_2N_2.HCl$ | 3*B* |
| 3. Beta diethylaminopropyl | $C_{18}H_{24}O_2N_2.HCl$ | 3*C* |
| 4. Beta di-*n*-butylaminopropyl | $C_{22}H_{32}O_2N_2.HCl$ | 3*D* |
| 5. Gamma diethylaminopropyl | $C_{18}H_{24}O_2N_2.HCl$ | 3*E* |
| 6. Gamma di-*n*-butylaminopropyl | $C_{22}H_{32}O_2N_2.HCl$ | 3*F* |
| 7. Beta, beta dimethyl $\gamma$-dimethylaminopropyl | $C_{18}H_{24}O_2N_2.HCl$ | 3*I* |
| **Esters of 4-amino-1-naphthoic acid** | | |
| 1. Beta diethylaminoethyl | $C_{17}H_{22}O_2N_2.HCl$ | 4*A* |
| 2. Beta di-*n*-butylaminoethyl | $C_{21}H_{30}O_2N_2.HCl$ | 4*B* |
| 3. Beta diethylaminopropyl | $C_{18}H_{24}O_2N_2.HCl$ | 4*C* |
| 4. Beta di-*n*-butylaminopropyl | $C_{22}H_{32}O_2N_2.HCl$ | 4*D* |
| 5. Gamma diethylaminopropyl | $C_{18}H_{24}O_2N_2.HCl$ | 4*E* |
| 6. Gamma di-*n*-butylaminopropyl | $C_{22}H_{32}O_2N_2.HCl$ | 4*F* |
| 7. Beta, beta dimethyl $\gamma$-dimethylaminopropyl | $C_{18}H_{24}O_2N_2.HCl$ | 4*I* |
| 8. Beta, beta dimethyl $\gamma$-diethylaminopropyl | $C_{20}H_{28}O_2N_2.HCl$ | 4*J* |
| **Esters of 5-amino-1-naphthoic acid** | | |
| 1. Beta-diethylaminoethyl | $C_{17}H_{22}O_2N_2.HCl$ | 5*A* |
| 2. Beta di-*n*-butylaminoethyl | $C_{21}H_{30}O_2N_2.HCl$ | 5*B* |
| 3. Beta diethylaminopropyl | $C_{18}H_{24}O_2N_2.HCl$ | 5*C* |
| 4. Beta di-*n*-butylaminopropyl | $C_{22}H_{32}O_2N_2.HCl$ | 5*D* |
| 5. Gamma diethylaminopropyl | $C_{18}H_{24}O_2N_2.HCl$ | 5*E* |
| 6. Gamma di-*n*-butylaminopropyl | $C_{22}H_{32}O_2N_2.HCl$ | 5*F* |
| **Ester of 6-amino-1-naphthoic acid** | | |
| 1. Beta diethylaminoethyl | $C_{17}H_{22}O_2N_2.HCl$ | 6*A* |
| **Control Compounds** | | |
| 1. Diethylaminoethyl-*p*-aminobenzoate | $C_{13}H_{20}O_2N_2.HCl$ | Procaine |
| 2. Methylbenzoylecgonine | $C_{17}H_{21}O_4N.HCl$ | Cocaine |
| 3. 4-Nitro naphthocaine HCl | $C_{17}H_{20}O_4N_2.HCl$ | 4-Nitro |

Table II.—Comparative Toxicity and Other Effects of Compounds Tested

| | Product | M. L. D., mg. per Gm. | Irritation | Blood Pressure Action | |
|---|---|---|---|---|---|
| 1. | 3*A* | 0.30 | Some in 0.5% Sol. | Transient depressor | 1% 3 cc. |
| 2. | 3*B* | 0.15 | None in 0.2% Sol. | Very slight depressor | 1% 2 cc. |
| 3. | 3*C* | 0.22 | Some in 0.1% Sol. | Slight depressor | 1% 2 cc. |
| 4. | 3*D* | 0.35 | Serious in 0.05% Sol. | Slight pressor | 1% 2 cc. |
| 5. | 3*E* | 0.18 | None in 0.3% Sol. | No effect | 1% 3 cc. |
| 6. | 3*F* | 0.18 | None in 0.1% Sol. | Transient depressor | 1% 3 cc. |
| 7. | 3*I* | 0.25 | Some in 0.25% Sol. | Slight depressor | 1% 3 cc. |
| 8. | 4*A* | 0.15 | None in 0.5% Sol. | Transient depressor | 1% 2 cc. |
| 9. | 4*B* | 0.18 | Serious in 0.5% Sol. | Very slight depressor | 1% 3 cc. |
| 10. | 4*C* | 0.09 | Some in 0.5% Sol. | Some depressor | 1% 2 cc. |
| 11. | 4*D* | 0.18 | Serious in 0.5% Sol. | Slight depressor | 0.5% 6 cc. |
| 12. | 4*E* | 0.10 | Slight in 0.5% Sol. | Slight depressor | 1% 2 cc. |
| 13. | 4*F* | 0.11 | None in 0.1% Sol. | Transient depressor | 1% 3 cc. |
| 14. | 4*I* | 0.18 | None in 0.25% Sol. | Slight depressor | 1% 2 cc. |
| 15. | 4*J* | 0.15 | None in 0.25% Sol. | Some depressor | 1% 3 cc. |
| 16. | 5*A* | 0.35 | Some in 0.25% Sol. | Some depressor | 1% 3 cc. |
| 17. | 5*B* | 0.35 | Some in 0.20% Sol. | Slight depressor | 1% 3 cc. |
| 18. | 5*C* | 0.18 | None in 0.5% Sol. | Some pressor | 1% 3 cc. |
| 19. | 5*D* | 0.80 | None in 0.5% Sol. | Very slight depressor | 1% 1 cc. |
| 20. | 5*E* | 0.18 | None in 0.25% Sol. | Very slight depressor | 1% 1 cc. |
| 21. | 5*F* | 0.20 | Some in 0.5% Sol. | More lasting depressor | 1% 2 cc. |
| 22. | 6*A* | 0.40 | None in 0.25% Sol. | Slight depressor | 1% 3 cc. |
| 23. | 4*A* as sulfamate | 0.15 | None in 0.5% Sol. | Transient depressor | 1% 3 cc. |
| 24. | Procaine | 0.45 | None in 0.5% Sol. | Transient depressor | 2% |
| 25. | Cocaine | 0.10 | None in 0.5% Sol. | Transient pressor | 1% |
| 26. | 4-Nitro | 0.30 | Some in 0.1% Sol. | Slight depressor | 1% |

trations up to 0.5% which makes them compare favorably in this respect with both cocaine and procaine. Nearly all of the series produce a moderate and transient depressor effect upon the blood pressure of a chloretonized dog with doses of 1 to 3 cc. of a 1% solution. This is similar to the effect of procaine but cocaine causes a transient pressor action.

From the toxicity standpoint three compounds 4*C*, 4*E* and 4*F* are as toxic as cocaine; 16 compounds, namely 3*A*, 3*B*, 3*C*,

*3E*, *3F*, *3I*, *4A*, *4B*, *4D*, *4I*, *4J*, *5C*, *5E*, *5F*, *4A* sulfamate and 4-nitro have M. L. D.'s from 0.15 mg. to 0.30 mg. per Gm. and are consequently less toxic than cocaine but more toxic than procaine; 5 compounds, namely, *3D*, *5A*, *5B*, *5D* and *6A* have M. L. D.'s from 0.35 mg. to 0.80 mg. per Gm. and are therefore comparable to procaine in toxicity.

As for local anesthetic efficiency (from data in Table III) nearly all of the compounds are quite effective by the rabbit cornea method (mucous membrane penetration) although *5D* was a notable exception (no effect with a 1% solution) and *3A*, *3I*, *5A*, *5C* are relatively weak. 4-Nitro is similar to cocaine by this method but is much too irritating. All the others proved stronger than cocaine by this method and cocaine is noted for its rapid and powerful local anesthetic action on mucous membranes.

By the frog sensory nerve method (to show local anesthesia by injection) *5D* is again ineffective in 1% solution while *3B*, *3C*, *3D*, *3E*, *3I*, *4B*, *5B*, *5E*, *5F* and 4-nitro are less effective than procaine or cocaine and *3A*, *3F*, *4A*, *4C*, *4D*, *4E*, *4F*, *4I*, *4J*, *5A*, *5C*, *6A* are fully as effective as cocaine by this method.

Only a few of the most promising compounds were tested by the human wheal or intradermal method. *3A* was found to be less active than procaine by this method while *4A* as the monohydrochloride and as the sulfamate was found to be fully as active as procaine.

Of all the compounds examined *4A* was definitely the most promising when judged by its comparative local anesthetic action; its freedom from local irritant action and the fact that it is only two-thirds as toxic as cocaine. It is also effective when given intraspinally as shown by a few tests upon rabbits and dogs. In producing anesthesia by injection it appeared to be fully as active as cocaine by the accepted methods and for mucous membranes it seemed to be definitely more powerful than cocaine, the pharmacological data obtained indicating that it is three times as active as this valuable local anesthetic.

The nitro compound from which this powerful ester is obtained chemically was found to be definitely less active—not over one-fifth as active by the frog sensory nerve method and not over one-third by the rabbit cornea method.

The stability of the *4A* ester in solution is quite good since a 0.1% solution without any preservative showed no appreciable loss in activity after two months at room temperature in a clear glass container.

Table III.—Local Anesthetic Efficiency of Compounds Tested

| | Product | Frog Sensory Nerve | Rabbit Cornea | Human Intradermal |
|---|---|---|---|---|
| 1. | *3A* | 1.0% = 2 minutes | 1.0% = 20 minutes | 0.4% = 20 minutes |
| | | 0.6% = 3 minutes | 0.75% = 12 minutes | 0.2% = 10 minutes |
| | | 0.4% = 5 minutes | 0.5% = 6 minutes | |
| 2. | *3B* | 2.0% = 4 minutes | 0.2% = 60 minutes | ..... |
| | | 1.5% = 5 minutes | 0.1% = 25 minutes | |
| | | 1.0% = 9 minutes | 0.05% = No effect | |
| 3. | *3C* | 1.0% = 4 minutes | 0.5% = 20 minutes | ..... |
| | | 0.5% = 7 minutes | 0.25% = 12 minutse | |
| | | | 0.10% = 5 minutes | |
| 4. | *3D* | 0.8% = 10 minutes | 0.05% = 5–10 min. | ..... |
| 5. | *3E* | 4.0% = 5 minutes | 0.3% = 25 minutes | ..... |
| | | 3.0% = 6 minutes | 0.2% = 20 minutes | |
| | | 2.0% = 7 minutes | 0.1% = 15 minutes | |
| 6. | *3F* | 1.0% = 3 minutes | 0.05% = 50 minutes | ..... |
| | | 0.5% = 5 minutes | 0.04% = 30 minutes | |
| | | 0.25% = 9 minutes | 0.03% = 10 minutes | |
| 7. | *3I* | 0.8% = 6 minutes | 0.5% = 15 minutes | ..... |
| | | 0.5% = 8 minutes | 0.25% = 5 minutes | |
| | | 0.4% = 10 minutes | | |
| 8. | *4A* | 0.5% = 1.8 minutes | 0.5% = 45 minutes | 0.20% = 20 minutes |
| | | 0.25% = 4 minutes | 0.15% = 30 minutes | 0.10% = 15 minutes |
| | | 0.10% = 7 minutes | 0.10% = 12 minutes | |
| | | | 0.05% = 6 minutes | |
| 9. | *4B* | 0.5% = 10–12 min. | 0.10% = 60 minutes | ..... |
| | | 0.4% = Over 15 min. | 0.05% = 20 minutse | |
| 10. | *4C* | 1.0% = 4 minutes | 0.10% = 35 minutes | ..... |
| | | 0.5% = 6 minutes | 0.05% = 20 minutes | |
| | | 0.25% = 8 minutes | 0.025% = 5 minutes | |

Table III (*Continued*)

| | Product | Frog Sensory Nerve | Rabbit Cornea | Human Intraderma |
|---|---|---|---|---|
| 11. | 4*D* | 1.0% = 3 minutes | 0.10% = 60 minutes | ..... |
| | | 0.5% = 5 minutes | 0.05% = 30 minutes | |
| | | 0.25% = 8 minutes | 0.02% = 10 minutes | |
| 12. | 4*E* | 1.0% = 2 minutes | 0.2% = 50 minutes | ..... |
| | | 0.5% = 4 minutes | 0.1% = 25 minutes | |
| | | 0.25% = 6 minutes | 0.05% = No effect | |
| 13. | 4*F* | 0.5% = 4 minutes | 0.05% = 45 minutes | ..... |
| | | 0.25% = 6 minutes | 0.04% = 25 minutes | |
| | | 0.15% = 12 minutes | | |
| 14. | 4*I* | 1.0% = 3 minutes | 0.10% = 30 minutes | ..... |
| | | 0.5% = 6 minutes | 0.05% = 15 minutes | |
| | | 0.25% × 9 minutes | 0.025% = 5 minutes | |
| 15. | 4*J* | 1.0% = 4 minutes | 0.10% = 45 minutes | ..... |
| | | 0.5% = 6 minutes | 0.05% = 25 minutes | |
| | | 0.25% = 8 minutes | 0.025% = Slight | |
| 16. | 5*A* | 0.5% = 3 minutes | 0.5% = 10 minutes | ..... |
| | | 0.2% = 6 minutes | 0.25% = 4 minutes | |
| | | 0.1% = 11 minutes | | ..... |
| 17. | 5*B* | 2.0% = 5 minutes | 0.5% = 30 minutes | |
| | | 1.0% = 15 minutes | 0.25% = 20 minutes | |
| | | | 0.10% = 5 minutes | |
| 18. | 5*C* | 0.5% = 2 minutes | 0.5% = 12 minutes | ..... |
| | | 0.2% = 7 minutes | 0.25% = 4 minutes | |
| | | 0.1% = 9 minutes | | |
| 19. | 5*D* | 1.0% = No effect | 1.0% = No effect | ..... |
| 20. | 5*E* | 0.8% = 4 minutes | 0.5% = 40 minutes | ..... |
| | | 0.5% = 5 minutes | 0.25% = 10 minutes | |
| | | 0.25% = 8 minutes | 0.10% = 5 minutes | |
| 21. | 5*F* | 2.0% = 4 minutes | 0.25% = 25 minutes | ..... |
| | | 1.0% = 7 minutes | 0.15% = 15 minutes | |
| | | 0.5% = 10 minutes | 0.10% = 9 minutes | |
| 22. | 6*A* | 0.5% = 3 minutes | 0.5% = 30 minutes | ..... |
| | | 0.25% = 6 minutes | 0.25% = 8 minutes | |
| | | 0.15% = 8 minutes | | |
| 23. | 4*A* | 1.0% = 2 minutes | 0.5% = 45 minutes | 0.10% = 15 minutes |
| | Sulfamate | 0.5% = 4 minutes | 0.2% = 30 minutes | 0.05% = 8 to 10 min- |
| | | 0.25% = 6 minutes | 0.1% = 20 minutes | utes |
| | | | 0.05% = 10 minutes | |
| 24. | Procaine | 1.0% = 3 minutes | 2.0% = 20 minutes | 0.10% = 15 minutes |
| | | 0.5% = 5.5 minutes | 1.0% = 15 minutes | |
| | | 0.5% = 11 minutes | 0.5% = About 3 min. | |
| 25. | Cocaine | 0.5% = 2.5 minutes | 0.5% = 16 minutes | 0.05% = 15 minutes |
| | | 0.25% = 9 minutes | 0.25% = 10 minutes | |
| | | 0.10% = 14 minutes | 0.10% = Negative | |
| 26. | 4-Nitro | 2.0% = 5.5 minutes | 0.5% = Too irritating | ..... |
| | | 1.0% = 8 minutes | 0.25% = 10 minutes | |
| | | 0.5% = 11 minutes | 0.10% = Negative | |
| 27. | 4*A* 0.1% Sol. 2 months old | 0.1% = 10 minutes | 0.10% = 11 minutes | ..... |


## SUMMARY

1. Twenty-two esters of amino-naphthoic acid have been examined for local anesthetic action, relative toxicity and irritant action in comparison with cocaine and procaine.

2. Nearly all of these esters compare favorably with cocaine in local anesthetic action both by injection and by topical application to mucous membranes but the 4*A* ester was the outstanding member of the series. It was found to be fully as active as cocaine by injection and about three times as powerful in anesthetizing mucous membranes as well as somewhat less toxic to white mice than cocaine and non-irritating in its effective dilutions.

3. The 4*A* ester is effective intraspinally in rabbits and dogs and is reasonably stable to room temperature in dilute (0.1%) solution.

4. The nitro compound from which the 4*A* ester is derived is definitely less effective than the ester itself and produces local irritation while the 4*A* ester does not.


## REFERENCES


(1) Blicke and Parke, *J. Am. Chem. Soc.*, 61 (1939), 1200.

(2) Fisk and Underhill, *J. Pharmacol.*, 49 (1933), 329.

(3) Sergievskava and Nesvad'ba, *J. Gen. Chem.* (U. S. S. R.), 8 (1938), 924; *Chem. Abst.*, 33 (1939), 1307.

(4) Sollmann, *J. Pharmacol.*, 11 (1918), 1, 17.

(5) Sollmann, *Ibid.*, 11 (1918), 69.

# A Pharmacological Study of the Active Principle of Passiflora Incarnata*

*By George H. Ruggy† and Clayton S. Smith*

In a previous paper, the authors have reported a chemical study of a physiologically active principle in *Passiflora incarnata*. No definite conclusions were drawn as to the chemical nature of this substance but it was isolated in relatively pure form as a mercury derivative. In this paper a pharmacological study of the active principle will be reported.

Other investigators (1, 2, 3) have reported certain activities of the crude Passiflora extracts, but the results are inconclusive. Therefore, because of the long use of this drug in medicine it was decided to make a rather extensive pharmacological study, using both the crude fluidextract and the active principle which has been isolated as a mercury derivative.

For this purpose an aqueous solution of the mercury derivative was prepared and the mercury was removed by saturating the solution with hydrogen sulfide. After filtering off the mercuric sulfide, the excess hydrogen sulfide was removed from the filtrate by aerating and heating below 60° C. The final volume was adjusted so that the concentration of active principle was that represented by 50 mg. of the mercury derivative per cc. This solution was used in the pharmacological experiments to be reported.

## EXPERIMENTAL

### A. GENERAL SYSTEMIC ACTION

In order to check the observations of other workers, a fluidextract of the crude drug was employed in addition to the solution of the active substance described above.

Alcohol was removed from the fluidextract by vacuum distillation and the aqueous residue was made up to volume with water. Doses of the alcohol-free extract equivalent to 2.5 Gm. to 60 Gm. of the crude drug were administered intravenously, intraperitoneally and orally to frogs, guinea pigs, rabbits, cats and dogs. A series of three to six intact animals was used in each case. Observations were made immediately and at the end of 30 minutes, 1 hour, 2 hours, 6 hours, 12 hours and 24 hours. No diminution of either voluntary or reflex activity could be demonstrated in any of the animals tested. A similar series of experiments was carried out using the solution of the active principle prepared as described. A fleeting increase in respiration was observed in those animals who received intravenous injections. Aside from this minor action no other effects were observed. These findings are of particular interest in view of the fact that the use of Passiflora in modern therapeutics is based solely on its supposed sedative action.

### B. ACTION ON THE CARDIOVASCULAR SYSTEM

The most intensive study was that made on the cardiovascular system. The carotid blood pressure was recorded to observe the effect of the active substance alone and in conjunction with other drugs having known effects.

*1. Active Substance Alone.*—Doses of 1–3 mg. per Kg. body weight were injected into the femoral vein of dogs anesthetized with ether. The carotid blood pressure was recorded in the usual manner. Immediately following the injection the blood pressure fell 40–60 mm. and rose slowly to normal. A typical reaction is shown in Fig. 1.

*2. Active Substance in Conjunction with Other Drugs.*—In an attempt to locate the site of action of the active principle its effects were studied following the administration of nicotine, atropine, adrenaline and pituitrin.

(*a*) *Nicotine:* A 0.4 per cent solution of nicotine was administered in 0.25-cc. doses until the heart no longer responded to vagal stimulation and the pupil no longer reacted to stimulation of the preganglionic fibers of the superior cervical ganglion. If the active substance was then injected, the usual fall in blood pressure occurred. This result indicates that if the action of the drug is through autonomic nerves its site of action is postganglionic.

(*b*) *Atropine:* A 1:1000 solution of atropine sulfate was administered until vagal stimulation no longer produced any effect on the blood pressure. Both vagi were then sectioned. The active principle was administered and a fall in the blood pressure similar to that observed without atropine was recorded (Fig. 2). This result indicates that if the substance acts on the parasympathetic division, the action is beyond the myoneural junction.

(*c*) *Adrenalin:* It was shown that adrenalin and the active substance were mutually antagonistic when administered consecutively. When administered together both a rise and fall in blood pressure occurred, one after the other. The rise occurred, first, if the adrenalin were present in the larger amount and, second, if the active substance were present in the larger amount. If the two were administered in amounts judged to be pharmacologically equivalent both a rise and fall occurred but it was not possible to predict which would occur first. No graphs showing these phenomena are included.

* From the Laboratory of Pharmacology, College of Medicine, The Ohio State University

† Presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

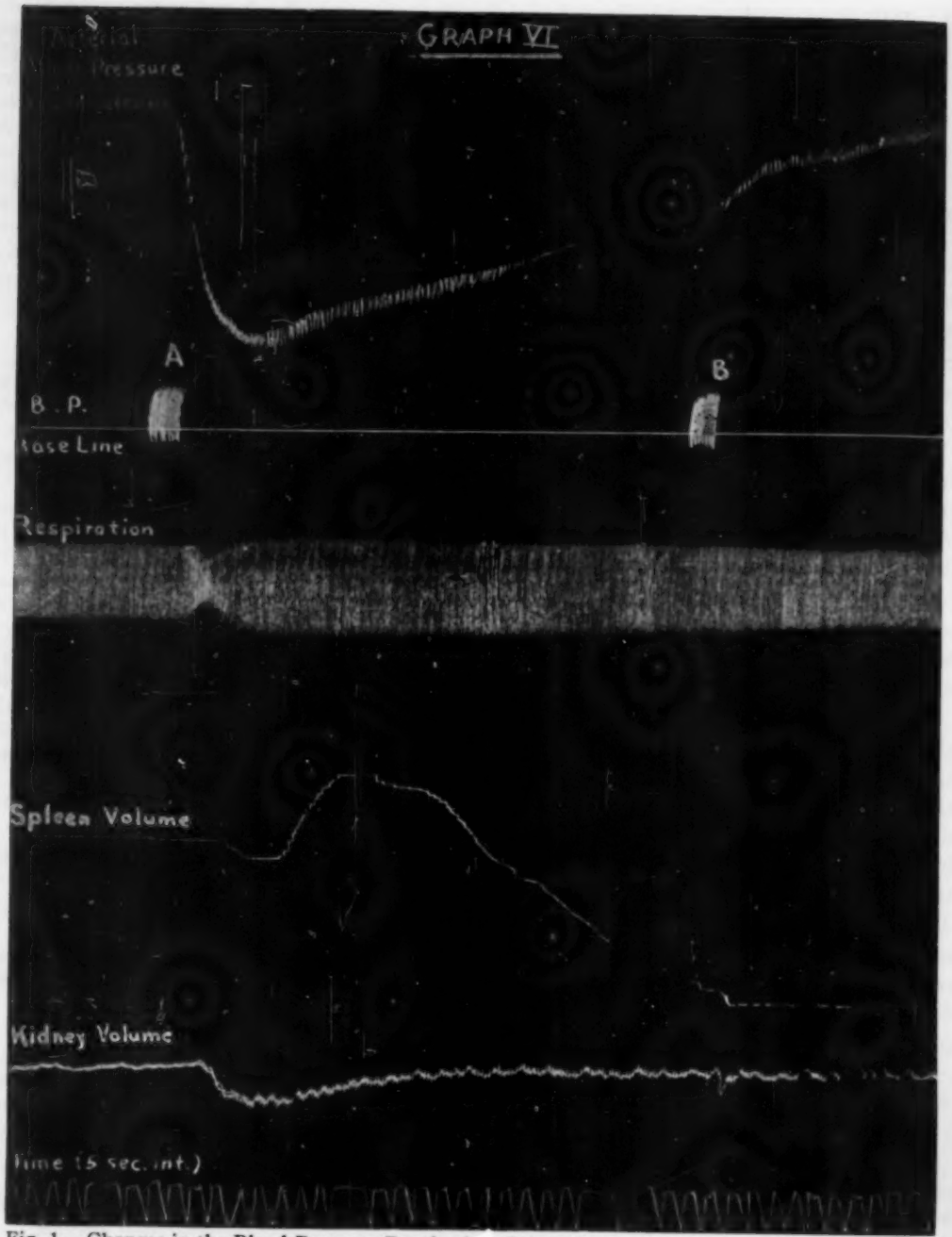


Fig. 1.—Changes in the Blood Pressure, Respiration, Spleen Volume and Kidney Volume Following the Injection of the Active Principle.

A. Injection of the active principle.
B. Stimulation of the peripheral end of the vagus nerve.

(*d*) *Pituitrin:* One cc. of solution of posterior pituitary (U. S. P.) was administered intravenously and at the peak of the rise in blood pressure the active substance was administered. A fall in blood pressure occurred at once. No graphs are included.

*3. Action on the Heart.*—The effect of the active principle on the heart was studied by means of myocardiograms and electrocardiograms. The myocardiogram showed no change in heart rate but did show that there was dilation of the heart. This was interpreted as being due to more complete filling compensating for the lowered blood pressure. The dilatation followed the fall in blood pressure and disappeared upon the return of the blood pressure to normal. The electrocardiogram revealed no changes in conduction but the conditions of the experiment were such that its interpretation was difficult.

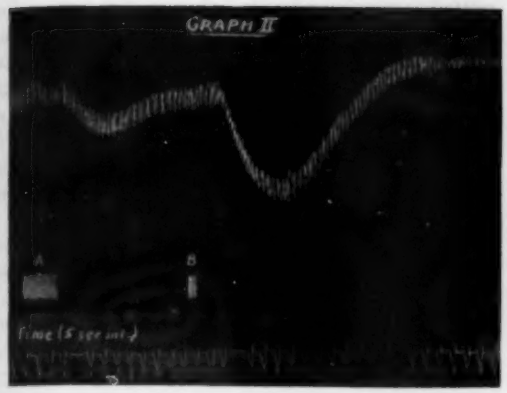


Fig. 2.—Arterial Blood Pressure. Action of the Active Principle in Conjunction with Atropine.
A. Stimulation of Peripheral Vagus.
B. Injection of 1 cc. Active Principle.

*4. Portal Injection.*—The substance is inactive by mouth, and when injected intravenously its action is fleeting. It was assumed for these reasons that the active principle was rapidly destroyed, possibly in the liver.

In order to test this assumption, injections of the active substance were made into the portal vein of dogs. The minimum dose necessary to produce a 30–40 mm. fall of the blood pressure when injected into the femoral vein was first determined for each animal tested. This dose was then injected into the portal vein, but no change in blood pressure resulted. The dose was increased gradually without effect. If 10 times the minimum dose was injected rapidly into the portal vein, a fall in the blood pressure of 10 ± 5 mm. occurred. From these experiments it was concluded that the active substance exerts only a fleeting action because it is destroyed in the liver.

One of two conclusions with regard to the site of action of the drug may be drawn from the experiments on the cardiovascular system: first, that it is a parasympathomimetic drug acting beyond the myoneural junction, and, second, that it acts directly upon the smooth muscle of the vessel walls to produce vasodilation.

### C. ORGAN VOLUME CHANGES

The spleen and kidney were placed in oncometers and were arranged to record changes in volume simultaneously with the recording of the blood pressure. Spleen volume increased greatly and kidney volume decreased (Fig. 1). This result indicated visceral vasodilation. There is some evidence obtained by a leg plethysmograph that peripheral vasodilation also occurred.

### D. ACTION ON THE RESPIRATORY SYSTEM (DOG)

Respiration was usually recorded as well as blood pressure. As the blood pressure fell rapidly (Fig. 1), the depth and rate of respiration increased. As the blood pressure arose the respiration returned to normal. DeNito's (2) conclusion that this respiratory effect was due to reflex stimulation of the respiratory center through the carotid sinus seems reasonable since a diminution of the blood pressure does set up respiratory changes probably as a result of concomitant anoxemia of the brain and stimulation of the carotid sinus. Since these respiratory changes were so closely allied to the blood pressure changes and since well-tried theories could explain the respiratory effects on the basis of a decrease in blood pressure, it was thought unnecessary to investigate these changes further.

### E. ACTION ON ISOLATED ORGANS

*1. Intestinal Strip (Rabbit).*—A short piece of the jejunum was placed in a bath of oxygenated Locke's solution at 37–38° C. A modification of the apparatus described by Sollman (4) was used for recording smooth muscle contraction. A normal record of the longitudinal contractions was made and then 1 cc. of unknown solution per 100 cc.

Fig. 3.—Isolated Intestinal Strip. Action of the Active Principle in Conjunction with Adrenalin.
E. 0.25 cc. 1:1000 adrenalin.
F. 5.0 cc. active principle.
G. 0.25 cc. 1:1000 adrenalin.

of Locke's solution was added. A very slight increase in tonus, recorded as a rise in the base line, occurred. The bath was changed and 3 cc. of unknown per 100-cc. Locke's solution were added. A slightly greater increase in tonus occurred. Neither the amplitude of the contractions nor the rhythmicity of contraction changed. Apparently the whole effect was one of increased tonus. Recovery was complete in about five minutes.

In an effort to check the site of action of the active substance on the intestinal strip a series of experiments was carried out using the unknown in conjunction with adrenalin. Using the set-up described above and fresh strips of jejunum, a normal record was secured and a check made to be sure that the unknown was exerting the effect previously observed. The Locke solution was then changed and 0.25 cc. of a 1:1000 adrenalin solution was added. Contractions stopped immediately. After about thirty seconds had elapsed, 5 cc. of the solution of active substance were added and within 20 seconds contractions were resumed. The reaction produced was typical in that the tonus increased above normal and then returned to normal. In order to be sure that normal recovery from the effects of adrenalin would not have begun just at the time of exposure to the active substance, another series of tests was run in which the muscle was permitted to remain under the influence of the adrenalin for varying periods of time before the addition of the active substance. The results of a typical test which was allowed to run for about three minutes are shown in Fig. 3. Similar experiments were performed using atropine and the active substance with almost identical results as those obtained with adrenalin. No graphs are included of the action with atropine.

*2. Uterus.—(a) Guinea pig:* Isolated uterine horns of virgin animals were studied using the same set-up as was employed for the intestinal strips. A normal record was obtained and then 3 cc. of the solution of active substance per 100 cc. of Locke's solution were added. An immediate and sharp contraction occurred (Fig. 4) raising the base line enormously. Rhythmicity of contraction remained practically unchanged but the amplitude was markedly diminished. The solution was now changed and adrenalin added. A markedly diminished activity followed but when the active substance was added a sharp contraction followed at once. The results obtained with the guinea-pig uterus were almost identical with those obtained with the intestinal strip.

*(b) Rabbit:* Similar experiments were made using the uterine horns of virgin rabbits. The uteri of three rabbits were used. All of these uteri re-

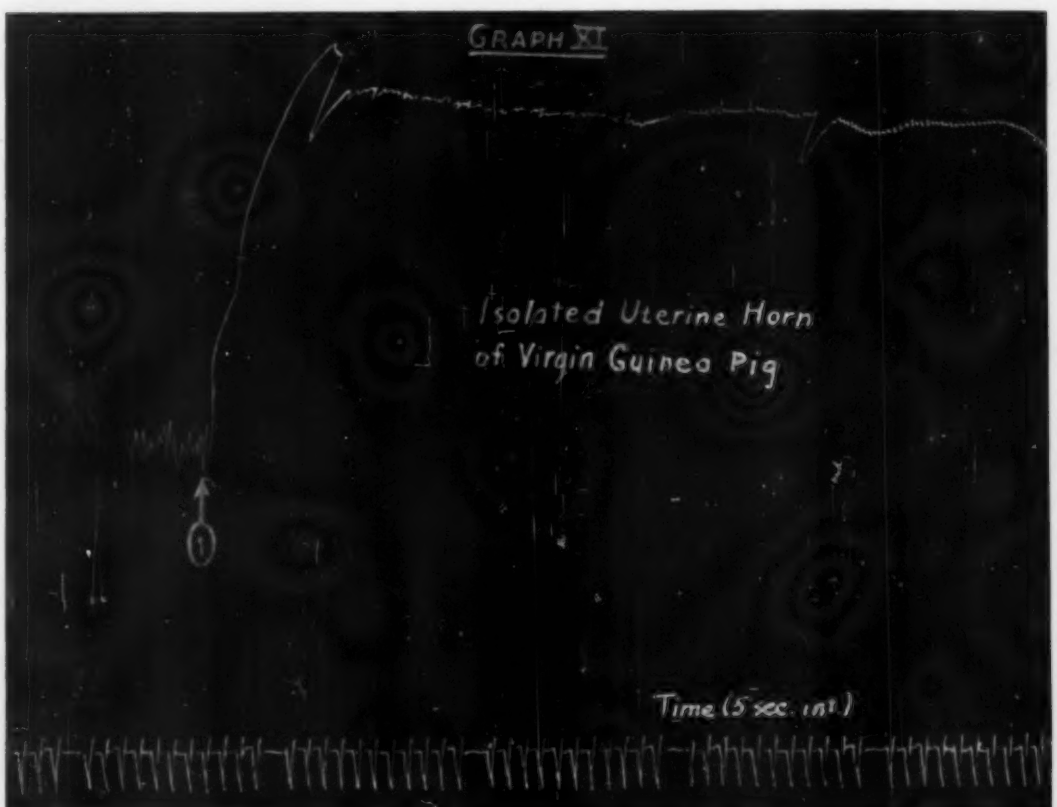


Fig. 4.—Response of Guinea-Pig Uterus to the Active Principle.
1. Addition of 3 cc. of solution to 100 cc. Locke Solution.

acted to adrenalin with a strong contraction. On the rabbit uterus the active substance acts in the same way as adrenalin. No graphs are included.

*3. Eye (Cat, Rabbit, Dog).*—The solution of active substance was instilled into the conjunctival sac of the cat, rabbit and dog. Time of exposure to the solution was varied as well as the lighting conditions. Absolutely no effects on the pupil were noted and no impairment of accommodation resulted as far as could be determined.

### DISCUSSION

The effect of the active principle in producing a fall in blood pressure, dilatation of the heart and constriction of the smooth muscle of the intestine would seem to indicate that the active principle was parasympathotropic. However, this is not universally true. For example, there was no action on the pupil and at no time was any increased salivation observed although no special experiments were done to check this point. Furthermore, the undiminished activity after vagotomy, nicotine and atropine argue against a parasympathetic mediation. The action of the drug in causing contractions of virgin uteri regardless of the previous action of adrenalin on those uteri suggested a musculotropic action.

The assumption of musculotropic action means that the substance must act on smooth muscle in different locations in opposite fashion, that is, to relax the muscle of the vessel walls and constrict that of the uterus and intestine. This seems unusual but it can be pointed out that synephrine and one or two other closely related sympathomimetic compounds have similar paradoxical actions and these drugs have been shown almost surely to be musculotropic (5).

### SUMMARY

1. A pharmacological study of a physiologically active compound from *Passiflora incarnata* has been made.

2. Neither the active substance itself nor the fluidextract of the crude drug had any action which could be construed as sedative.

3. The active substance caused lowering of the blood pressure and contraction of smooth muscle of the gut and uterus.

4. The activity of the substance has been shown to be unaffected by vagotomy, atropine, nicotine or pituitrin.

5. The drug probably exerts its characteristic activity by direct action on smooth muscle.

### REFERENCES

(1) Ott, *Medical Bulletin*, 29 (1898), 457.

(2) DeNito, *Rassegna di Terapia e Patologia Clinica*, 3 (1931), 193.

(3) Fellows and Smith, JOUR. A. PH. A., 28 (1938), 565.

(4) Sollmann and Hanzlik, "Experimental Pharmacology," Saunders (1938), page 145.

(5) Tainter and Seidenfeld, *J. Pharmacol.*, 40 (1930), 23.

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# Toxicity of Selenium-Cystine and Some Other Organic Selenium Compounds*

*By Alvin L. Moxon*†

Earlier studies on the selenium problem (1, 2, 3) have demonstrated that the toxicity as well as the selenium is carried in the protein fraction of seleniferous grains. The selenium is thought to occur in organic combination (4, 5) and it has been postulated that selenium could be replacing sulfur in the amino acids: cystine and methionine (6). Jones, Horn and Gersdorf (5), by a special enzymatic hydrolysis of gluten from seleniferous wheat, have isolated two fractions which contained practically all of the selenium and most of the cystine. The remaining fractions were practically free from selenium and contained only traces of cystine.

The toxicity of a number of organic selenium compounds has been investigated (7) and it was found that the toxicity of the selenium in the particular compounds studied did not approach the toxicity of sele-

* The author wishes to thank Dr. Arne Fredga of Uppsala University, Uppsala, Sweden, for the generous supply of selenium-cystine and other organic selenium compounds, and Dr. C. A. Elvehjem, Dept. of Agricultural Biochemistry, University of Wisconsin, for courtesies and helpful suggestions during the course of the work. The work reported herein was done in the laboratories of the Department of Agricultural Biochemistry, University of Wisconsin, during a leave of absence from the South Dakota State College.

† Chemist, S. Dak. Agr. Expt. Station, South Dakota State College, Brookings, South Dakota.

nium in inorganic selenites or selenates or in seleniferous grains. It has been shown (8) that inorganic selenium (selenites and selenates) and naturally occurring selenium in the toxic grains are about equal in toxicity when orally ingested. Furthermore, it has been observed that, by intraperitoneal injection, inorganic selenium is several times more toxic than selenium in organic combination in such compounds as $\beta,\beta'$-diselenodipropionic acid (7).

Gordon (9) attempted to synthesize the selenium analog of cystine but failed to isolate the pure compound. Fredga (10), however, has been successful in synthesizing selenium-cystine and has been very generous in furnishing the author with a supply of this selenium-substituted amino acid and several other organic selenium compounds.

## EXPERIMENTAL

A solution containing 0.5 mg. of selenium per cc. was prepared by dissolving the required amount of selenium-cystine in 50 cc. of distilled water to which had been added 3 cc. of $N/10$ HCl.

The required amount of solution was injected intraperitoneally into albino rats weighing 100 to 150 Gm. The intraperitoneal route of injection was chosen because it simulates oral ingestion of the toxic agent more closely than does intravenous or subcutaneous injection.

A solution containing 3 cc. of $N/10$ HCl per 50 cc. was injected into control animals and no toxic effects were noted when amounts equal to twice the volume of selenium-cystine solution were injected.

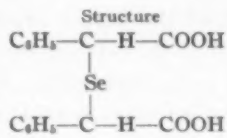
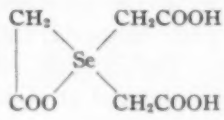
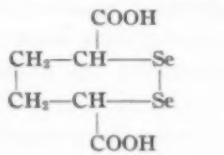
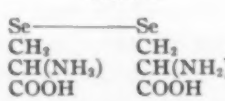
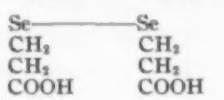
Table I.—Toxicity of Selenium-Cystine

| Dosage Data | Mg. Se per Kg. Body Weight |
|---|---|
| Range of dosage used | 3.0–30.0 |
| Lowest dosage to cause death | 3.50 |
| Highest dosage which failed to cause death in 48 hours | 4.25 |
| Dosage killing 100% in 48 hrs. | 4.5 |
| Dosage killing 75% in 48 hrs. (M. F. D.) | 4.0 |
| Total number of rats injected | 65 |

The minimum fatal dose (M. F. D.) was taken as the minimum amount of selenium (mg. per Kg. of body weight) to cause death in 75 per cent of the rats within two days (11). According to the results obtained (Table I) the minimum fatal dose of selenium in the form of selenium-cystine is 4.0 mg. per Kg. body weight which compares very favorably with the toxicity of selenium in the form of $Na_2SeO_3$ or $Na_2SeO_4$ (11). Since selenium-cystine contains 47.37 per cent of selenium the minimum fatal dose of selenium-cystine would be $2.11 \times 4.0$ or 8.44 mg. per Kg.

The toxicities of three other organic selenium compounds, generously supplied by Dr. Fredga, were also investigated and the results are given in Table II. These compounds[1] are much less toxic than selenium-cystine. It is of interest to compare the toxicities and structures of selenium-cystine and the other organic selenium compounds, especially the $\beta,\beta'$-diselenodipropionic acid (7).

Table II.—Comparison of Toxicity and Structure

| Compound | Structure | Toxicity (M. F. D.), mg./Kg. |
|---|---|---|
| Selenium-diphenyl acetic acid (meso form) |  | Over 30 |
| Dimethyl-selenetin-dicarboxylic acid |  | Over 30 |
| Tetrahydro-selenophen $\alpha,\alpha'$-dicarboxylic acid (racemic vorm) |  | Over 30 |
| Selenium-cystine (optically inactive) |  | 4.0 |
| $\beta,\beta'$-Diselenodipropionic[2] acid |  | 25–30 (7) |

Work under way at the present time indicates that selenium-cystine when administered orally is almost equal in toxicity to inorganic selenium ($Na_2SeO_3$ and $Na_2SeO_4$) and to naturally occurring selenium in toxic grains. This work will be reported later.

## DISCUSSION

The results indicate that selenium-cystine is much more toxic than any other organic selenium compound which has been investigated to date (7, 12, 13). The toxicity of the selenium in selenium-cystine as shown in Table III is approximately seven times that of selenium in the form of $\beta,\beta'$-diselenodipropionic acid.

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[1] For details on preparation and properties of these compounds, other than selenium-cystine, see "Studien uber Selen-Di-Karbon sauren und Diselen-Di Karbonsauren," Arne Fredga, Uppsala Universitets Arsskrift (1935) 5, 230 pages.

[2] Prepared by E. Page Painter, Division of Agricultural Biochemistry, University of Minnesota, St. Paul, Minnesota.

When injected intraperitoneally the toxicity of selenium in the form of selenium-cystine is approximately equal to the toxicity of selenium in sodium selenate or sodium selenite. The toxicities of the selenium in the two, above mentioned, inorganic compounds are in the same range as the toxicity of selenium in seleniferous grains. This has been indicated by oral administration of selenium-cystine.

SUMMARY

The minimum fatal dose of selenium in the form of selenium-cystine when injected intraperitoneally into albino rats was found to be 4.0 mg. per Kg. of body weight. This is equivalent to 8.44 mg. of selenium-cystine per Kg. of body weight.

REFERENCES

(1) Franke, K. W., *J. Nutrition*, 8 (1934), 609–613.

(2) Franke, K. W., and Painter, E. P., *Cereal Chem.*, 13 (1936), 67–70.

(3) Horn, M. J., Nelson, E. M., and Jones, D. B., *Ibid.*, 13 (1936), 126–139.

(4) Painter, E. P., and Franke, K. W., *J. Biol. Chem.*, 111 (1935), 643–651.

(5) Jones, D. B., Horn, M. J., and Gersdorf, C. E. F., *Cereal Chem.*, 14 (1937), 130–134.

(6) Painter, E. P., and Franke, K. W., *Ibid.*, 13 (1936), 172–179.

(7) Moxon, A. L., Anderson, H. D., and Painter, E. P., *J. Pharmacol.*, 63 (1938), 357–368.

(8) Franke, K. W., and Painter, E. P., *Cereal Chem.*, 15 (1938), 1–24.

(9) Gordon, J. C., Dissertation, Catholic Univ. of Am. (1935), 43 pages.

(10) Fredga, A., *Svensk Kemisk Tidskrift*, 48 (1936), 160.

(11) Franke, K. W., and Moxon, A. L., *J. Pharmacol.*, 58 (1936), 454–459.

(12) Kondo, S., *Japan J. Med. Sci.*, IV Pharm. (1933), No. 1, 213–232.

(13) Kondo, S., *Japan J. Med. Sci.*, IV Pharm. 9 (1935), No. 1, 29.

NOTICE

Please use graph paper for illustrations as specified under "Notice to Authors."

# The Pharmacology of Soaps

## II. The Irritant Action of Soaps on Human Skin

*By Byron E. Emery and Leroy D. Edwards**

In a previous paper (1) the actions of soaps on human red cells and earthworm segments were reported. During the past year, this work has been extended by the study of the irritant action of sodium and potassium soaps made from chemically pure fatty acids on human skin.

The literature gives many conflicting reports as to the component of a soap solution responsible for its irritant action on skin, *i. e.*, free alkali (2), hydrolytic alkalinity (2), free fatty acid (3, 4), acid soap (3), unsaturated acids (5), neutral soaps (3), added ingredients—perfumes (6, 7), antiseptic substances (8, 9), etc. The oils most commonly mentioned as being capable of producing irritant soaps are cottonseed (2, 8), cocoanut (5, 8, 10) and palm (5). Many writers (2, 11, 12) discuss the probability of differences in skin as being a contributing factor to soap irritation. Practically all of these workers used some form of a patch or swab test to determine the results reported.

EXPERIMENTAL

*Method Employed.*—The patch test, cited above, usually consists of the application to the skin of solid soap or bits of paper or gauze impregnated with a soap solution (alcoholic or aqueous) for some definite period of time. This method is held to be deficient for the following reaons: (1) insoluble soap cannot produce a reaction, (2) a definite volume or a definite concentration of a soap solution cannot remain in contact with the skin, (3) the volume of soap solution is not large enough, (4) paper and gauze have electrical charges which affect the state of a colloidal soap solution, (5) the area of skin covered by a soap solution on paper or gauze is not constant. In order to avoid these difficulties the device as shown in Fig. 1 was developed for use in this study of the irritant action of soaps on human skin. The diaphragm rim was lubricated with a jeily of tragacanth, Irish moss, glycerin, boroglycerin and benzoic acid. Approximately 25 cc. of a soap solution were added to the diaphragm and strapped to the inner surface of the arm or leg.

* From the Department of Pharmacology, School of Medicine, and the School of Pharmacy, Western Reserve University.

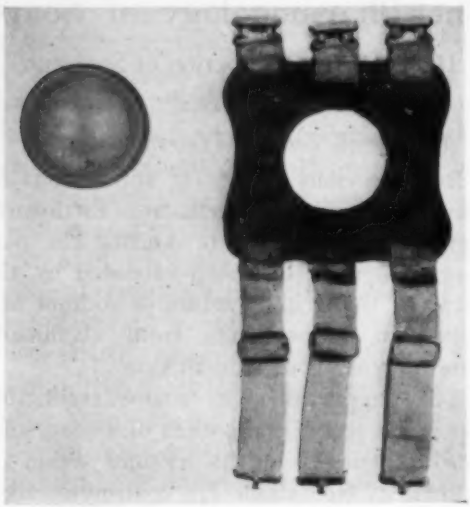

Fig. 1.—Device Used in Skin Tests.

The solution was removed after it had been in contact with the skin for four hours, the area washed with warm tap water, dried and examined at various intervals for signs of irritation. If there was irritation five minutes after the conclusion of the test and this irritation remained for more than two hours (irritation was determined by the presence of redness, itching, pain or any other damage to the epithelium), the result was recorded as "Plus," "Plus-Minus" if irritation was present but disappeared in two hours and "Minus" if no irritation was noted. No detectable differences were observable between the sensitivity of the inner surface of the leg and that of the arm.

In all reports reviewed, the concentrations of the soap solutions employed were expressed in per cent. In this work, in order to put the test on a more scientific basis, an equivalent molarity of each soap has been used. Calculating back from a sodium soap mixture containing 20 to 25% of cocoanut oil, and assuming a lather concentration of 5%, the approximate concentration of sodium laurate in the lather would be 0.5% which is equivalent to 0.0225*M* or 0.0225*N*. Since this concentration of sodium laurate was found to be very irritating to the skin over a four-hour period of time, 0.0225*N* solutions were used for the comparison of the irritant actions of the salts of the pure fatty acids studied. This concentration is further ideal in that soap solutions of this normality exhibit true soap properties and, in addition, such a concentration is above the critical concentration level for all of the salts except for caprylic and capric acids.

In the conduction of $p_H$ control tests on skin it was found necessary to use alkaline buffer salts to maintain the desired hydrion level equivalent to soap solutions because of the neutralization capacity of the skin. Water—$p_H$ 5.8, 0.0225*N* $Na_2HPO_4$—$p_H$ 8.8, 0.0225*N* $Na_2CO_3$—$p_H$ 10.9, 0.0225*N* $K_2HPO_4$—$p_H$ 8.7, 0.0225*N* $K_2CO_3$—$p_H$ 10.4, gave no evidence of irritation in tests carried out on 24 males and 14 females.

The chemically pure fatty acids, *n*-caprylic, *n*-capric, lauric, myristic, palmitic and stearic, all solid acids at room temperature except *n*-caprylic, were obtained from the Eastman Kodak Company. Linoleic, oleic and ricinoleic, all liquid acids at room temperature, were obtained from Schering-Kahlbaum. The purity of these acids was assumed to be sufficient to indicate possible differences in irritant properties of the resultant soaps. The sodium soaps of all the fatty acids were prepared by adding 0.1 cc. less than the calculated amount of 1*N* NaOH to the acid dissolved in previously neutralized 95% alcohol. The remaining alkali as 0.1*N* NaOH was added dropwise until a drop of the alcoholic soap solution produced a pink color with phenolphthalein. The alcohol was distilled from the soap under reduced pressure, and the powdered soap dried for three hours under vacuum at a temperature of 105° C. The potassium soaps were prepared by the same method.

*Results of Soap Tests on Skin.*—The sodium and potassium soaps of the above acids were studied as previously outlined. The results given in Fig. 2 were obtained on 24 males and 14 females. A larger number of individuals would, of course, be desirable. When the soaps are compared as to their greatest irritant action on skin, the acids may be arranged in order of decreasing toxicity as follows:

*Sodium Soaps:* Females—lauric, myristic, linoleic, *n*-capric, oleic, ricinoleic, palmitic, *n*-caprylic. (No reaction: stearic.) Males—lauric, myristic, oleic, *n*-capric, linoleic, ricinoleic, oleic. (No retion: *n*-caprylic, palmitic and stearic.) Combined males and females—lauric, myristic, linoleic, *n*-capric, oleic, ricinoleic, palmitic, *n*-caprylic. (No reaction: stearic.)

*Potassium Soaps:* Females—lauric, myristic, linoleic, *n*-capric, *n*-caprylic, oleic, palmitic, stearic, ricinoleic. Males—lauric, myristic, linoleic, oleic, *n*-caprylic, palmitic, *n*-capric, ricinoleic. (No reaction: stearic.) Combined male and female—lauric, myristic, linoleic, *n*-capric, oleic, *n*-caprylic, palmitic, stearic, ricinoleic.

When both the potassium and sodium soaps, as per the combined male and female results, are arranged according to decreasing toxicity, the order is as follows: K laurate, Na laurate, K myristate, Na myristate, Na linoleate, K linoleate, Na *n*-caprate, Na oelate, Na ricinoleate, K *n*-caprate, K oleate, K *n*-caprylate, K palmitate, K stearate, Na palmitate, K ricinoleate, Na *n*-caprylate. (No reaction: Na stearate.)

## DISCUSSION OF RESULTS

The graphic method of presentation of the irritant properties and the chemical composition of soaps permits a comparison of the activity of one member of a homologous series with that of another. In the case of

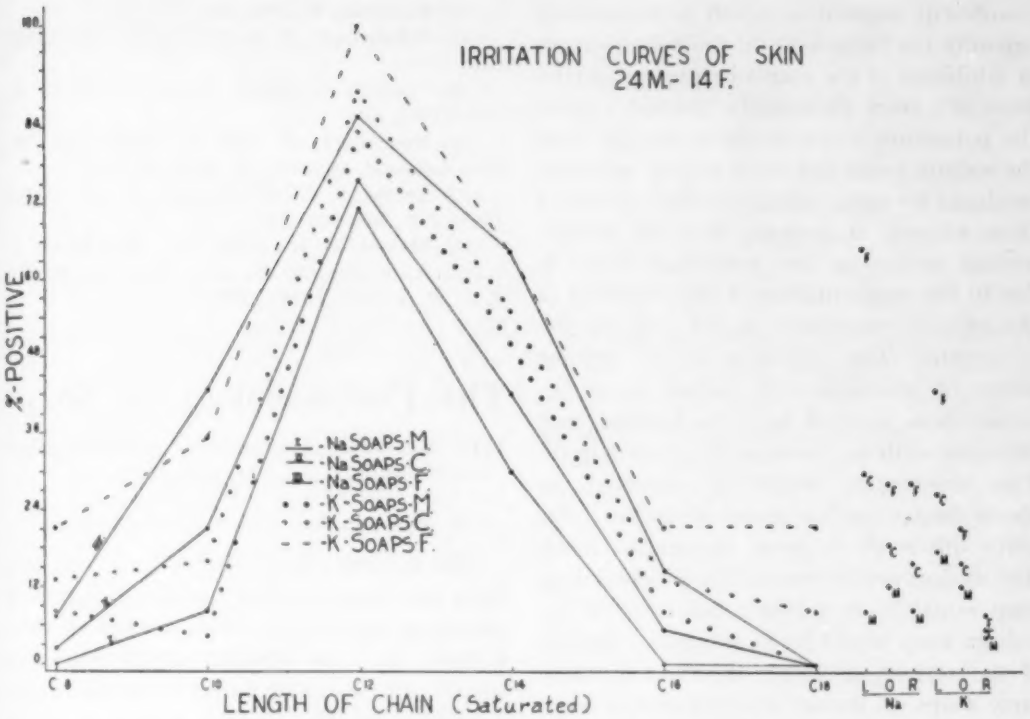


Fig. 2.—Curves of Irritant Action of Soaps on Human Skin.
All soap solutions were 0.0225*N*. % Positive—the per cent of individuals with irritant results. M—males; F—females; C—combined males and females. $\frac{LOR}{Na}$: Sodium soaps of linoleic, oleic and ricinoleic acids. $\frac{LOR}{K}$: Potassium soaps of linoleic, oleic and ricinoleic acids.

the unsaturated acids (linoleic, oleic and ricinoleic) only a single point of the irritation curve has been determined. It is immediately seen that the potassium soaps are more irritant than the sodium soaps on both females and males. Females are more readily irritated than males; hence, the combined curve falls intermediate between the male and female curves. The exceptions to these general statements are that the sodium and potassium soaps of oleic acid are equal in their irritant actions on males and females, and that the potassium soap of ricinoleic acid is less irritant than the sodium soap on both males and females. Lauric and myristic acids, by far, produce the most irritant soaps and are approached in magnitude only by the soaps of *n*-capric and linoleic acids. The left legs of the curves are true representations of the relative irritant properties of the low molecular weight salts. The right legs, however, may be determined in a large way by the low solubility of the high molecular weight salts. It should be pointed out that acids of the same number of carbon atoms (oleic, linoleic, stearic and ricinoleic) show marked differences in irritant properties. These differences cannot be explained on a basis of insolubility alone since ricinoleic and oleic soaps (both highly soluble) are very much less irritant than linoleic soaps (also highly soluble). Undoubtedly the presence of double bonds and substituent groups play an important rôle in determining the irritant properties of these soaps on human skin. Further proof for this statement is for the moment lacking.

The results of the alkali controls on the skin indicate that the hydrolytic alkalinity alone of a neutral soap in solution is not the cause of soap irritation. Another fact not to be overlooked, as noted by several other workers, is that the most irritant solutions, laurates and myristates, have a $p_H$ much lower than the less irritant solutions, stearates and palmitates. The effects of small quantities of excess alkali in a soap or an

insufficient amount of alkali to completely saponify the fatty acid on the enhancement or inhibition of the soap's irritant properties have not been thoroughly studied. Since the potassium soaps are more irritant than the sodium soaps and there was no irritation produced by either alkaline buffer control of these cations, it appears that the greater irritant action of the potassium soaps is due to the augmentation of the solubility of the irritant component of the soap by the potassium. The difference in the irritant action of potassium and sodium soaps becomes more marked with the laurates and increases with an increase in chain length. This observation would be expected, as above twelve carbon atom acids the solubility difference is great enough to mean that an appreciable amount of the potassium soap would be in solution but little of the sodium soap would be in solution. Studies of the actions of simple mixtures of these same soaps on human skin are now in progress.

### CONCLUSIONS

1. An improved patch test method for the determination of the irritant properties of soap solutions on human skin has been described.
2. The irritant effects of the potassium and sodium soap solutions of nine pure fatty acids common in soaps have been determined on human skin.
3. Lauric and myristic acids produce the most irritant soaps of the nine acids studied.
4. Potassium soaps of the saturated acids have been found to be more irritant than the corresponding sodium soaps.
5. Females are more subject to irritation from soap solutions than males.
6. Soap irritation is not due to hydrolytic alkalinity alone.

### REFERENCES

(1) Edwards, L. D., JOUR. A. PH. A., 28 (1939), 209.
(2) Gardiner, F., *Edinburgh Med. J.*, 8 (1912), 514.
(3) Siebert, C., *Med. Klinik*, 18 (1922), 1039.
(4) Blank, I. H., *Arch. Dermatol. Syphilol.*, 39 (1939), 811.
(5) Weber, K. I., *Seifenseider-Ztg.*, 64 (1937), 536.
(6) Luklinski, E., *Ibid.*, 50 (1923), 4.
(7) Schwartz, I., *U. S. P. H. S. Bull.*, 32 (1934), 215.
(8) "Queries and Minor Notes," *J. A. M. A.*, 106 (1936), 644.
(9) Rostenberg, A., and Sulzberger, M. B., *Arch Dermatol. Syphilol.*, 35 (1937), 433.
(10) Beedle, F. C., and Bolam, T. R., *J. Soc. Chem. Ind.*, 40 (1921) 25T.
(11) Mayers, M. R., *Safety Eng.*, 76 (1938) 9.
(12) Sulzberger, M. B., and Finnerud, C. W., *J. A. M. A.*, 111 (1938), 1528.

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# The Pharmacology of Soaps

## III. The Irritant Action of Sodium Alkyl Sulfates on Human Skin

*By Byron E. Emery and Leroy D. Edwards**

The sodium alkyl sulfates have recently been introduced on the market in forms of shampoos, washing powders, cosmetics, dentrifices, etc., as substitutes for ordinary soaps. Little can be found in the literature pertaining to the irritant action of these substances on human skin. Carpentar (1) reports irritation of the skin in the case of a hairdresser from daily contact with a "hymolal salt shampoo." Ammonium laurvl sulfate has been claimed to produce skin irritation by Biederman (2). Because of this lack of any definite information concerning the irritant actions of the alkyl sulfates, it was considered advisable to run skin tests similar to those outlined in the preceding paper for true soaps (3).

### EXPERIMENTAL

The sodium alkyl sulfates from C-8 to C-18, inclusive were supplied by the Procter & Gamble Company, Ivorydale, Ohio. The concentration of these sulfates employed in the tests was the same as that used in the tests of the pure soaps (0.0225*N*). Since in the commercial production of the alkyl sulfates it is common to add inorganic salts to enhance the detergent properties of these soap substitutes, additional tests were conducted after the addition of 0.002*N* NaCl, 0.002*N* $Na_2CO_3$, or 0.002*N* $Na_2SO_4$ to the 0.0225*N* solutions of the alkyl sulfates. The results presented here in Table I were obtained on the same 24 males and 14 females as were the results for the true soaps mentioned above. The per cent positive values for the sodium and potassium salts of the individual fatty acids are included for the

* From the Department of Pharmacology, School of Medicine, and the School of Pharmacy, Western Reserve University.

Table I.—Per Cent Positive Reactions of Alkyl Sulfates and True Soaps on Human Skin

(24 Males and 14 Females; Soap or Alkyl Sulfate Concentration, 0.0225*N*; NaCl, $Na_2CO_3$ and $Na_2SO_4$ Concentration, 0.002*N*)

| | C-8, Per Cent | C-10, Per Cent | C-12, Per Cent | C-14, Per Cent | C-16, Per Cent | C-18, Per Cent |
|---|---|---|---|---|---|---|
| Sodium alkyl sulfates | 5 | 5 | 43 | 24 | 5 | 0 |
| Sodium soap | 2 | 19 | 70 | 41 | 4 | 0 |
| Potassium soap | 13 | 15 | 89 | 54 | 13 | 10 |
| Sodium alkyl sulfates plus sodium chloride | 14 | 25 | 57 | 72 | 5 | 0 |
| Sodium alkyl sulfate plus sodium sulfate | 14 | 25 | 58 | 67 | 5 | 0 |
| Sodium alkyl sulfate plus sodium carbonate | 31 | 47 | 85 | 39 | 8 | 8 |

purpose of comparison. While the number of tests should be greater, the results should offer some idea as to the relative power of the different alkyl sulfates to cause skin irritation.

The values for the sodium alkyl sulfates are striking in that they show that the alkyl sulfates are much less irritant to the human skin than the sodium or potassium salts of the pure fatty acids. The results of the sulfates, however, are similar to the results of the others in that lauryl and myristyl derivatives possess the greatest irritant properties. With the addition of minute quantities of the salts of strong acids and strong bases, NaCl and $Na_2SO_4$, the power to produce irritation in every case except palmityl and stearyl sulfates is decidedly augmented. This increase in activity is pronounced with lower members of the series and reaches its maximum with sodium myristyl sulfate. Since there is the presence of a common ion in sodium which should give a diminution of the dissociation of the alkyl sulfates, the results suggest that the increased amount of the undissociated alkyl sulfate is responsible for the augmentation of the irritant action. Palmityl and stearyl sulfates do not respond because of their low solubility. In the case of the addition of an alkaline salt, $Na_2CO_3$, the results are somewhat different. Here the augmentation reaches its maximum with lauryl sulfate, and in the case of the lower members, it is much greater than the increase noted with NaCl or $Na_2SO_4$. Sodium myristyl sulfate in the presence of the carbonate was a jel while in the presence of the other salts it was not—this should explain the shift in the results. The high values for caprylyl, capryl and lauryl sulfates would be expected since there is not only the presence of a common ion but an increase in the alkalinity as well. Controls of the inorganic salts were negative.

## CONCLUSIONS

1. Pure sodium alkyl sulfates are less irritant to human skin than the pure sodium or potassium salts of the saturated fatty acids from C-8 to C-18.

2. Sodium lauryl sulfate is the most frequent cause of skin irritation of the series studied, but it is closely followed by myristyl sulfate.

3. Sodium chloride and sodium sulfate enhance the irritant action of these soap substitutes markedly. This enhancement is greater with sodium carbonate.

## REFERENCES

(1) Carpentar, C. C., *Arch Derm. & Syph.*, 30 (1934), 517.

(2) Biederman, J. B., *Pharm. Jour.*, 140 (1938), 77.

(3) Emery, B. E., and Edwards, L. D., Jour. A. Ph. A., 29 (1940), 251.

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# Iodocholeate*

## Its Efficiency as a Germicide and Its Clinical Performance

*By H. R. Scherzer and Paul Goedrich.*†

In the field of disinfection and bacterial control, inorganic iodine, despite its obvious disadvantages, has been used in alcoholic or aqueous solutions practically unchanged for more than a century. However, the extensive use of iodine is limited by its irritation, corrosiveness and other disturbing properties.

As to iodine's effectiveness as a germicide in comparison with modern antiseptics, Gershenfeld and Miller (1) stated in 1931: "From the standpoint of bactericidal action, 3% solutions of iodine were found to be superior to any of the other commonly used bactericidal agents in the dilutions most frequently employed." Nye (2) several years later confirmed in effect their findings.

In 1936 Goedrich brought forth a new iodine compound which was named Iodocholeate. This new compound, the result

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† 133 Heller Parkway, Newark, N. J.

Table I.—Bactericidal Performance

Organism: *Staphylococcus aureus;* 50% horse serum plus germicidal dilution; (1 hour allowed to elapse before running test); temperature 20° C.; method, F. D. A.

| Germicide | Dilutions | Contact Time 5 Min. | 10 Min. | 15 Min. | Phenol Coefficients of Available Iodine |
|---|---|---|---|---|---|
| Tr. Iodine U. S. P. 7.13% | 1/10 | − | − | − | Iodine in Tincture, 3.5 |
| | 1/15 | + | − | − | |
| | 1/20 | + | + | + | |
| Iodocholeate aqueous soln. 2.10% | 1/10 | − | − | − | Iodine in Iodocholeate, 10.0 |
| | 1/15 | + | + | − | |
| | 1/20 | + | + | + | |
| Tr. Iodine U. S. P. 7.00% | 1/10 | − | − | − | Iodine in Tincture, 3.6 |
| | 1/15 | + | − | − | |
| | 1/20 | + | + | + | |
| Iodocholeate (15% alcohol) 2.46% | 1/15 | − | − | − | Iodine in Iodocholeate, 11.8 |
| | 1/20 | + | + | − | |
| | 1/25 | + | + | + | |

of a partial chemical combination and partial adsorption of iodine with sodium glycocholeate and taurocholeate, as contained in natural bile, obviously overcomes all of the disadvantages of inorganic iodine. It is non-irritating, non-volatile and not as destructive to animal tissues as is inorganic iodine, and even its toxicity by comparison has been considerably reduced. Iodocholeate is easily soluble in water or alcohol. Two and five-tenths per cent solutions of this compound containing 35% alcohol were found to be physically stable and to maintain a permanently high germicidal action.

## EXPERIMENTAL

In order to have a basis of comparison of the germicidal action of Iodocholeate with popular proprietary as well as non-proprietary solutions which are ordinarily used as antiseptics, a technique outlined by Nye was followed which consisted of obtaining the minimum concentration necessary to kill a standardized strain of *Staphylococcus aureus* at 37° C. within 5 minutes. Nye's results, obtained by this technique, show that most of the germicides in popular use possess no bactericidal action after a dilution of 1:16. The Compound Solution of Iodine U. S. P. 5% displayed the best performance in his series, showing a killing action in a dilution of 1:256. However, the Iodocholeate 5% aqueous solution, under identical conditions, even exceeded this action, for it displayed a germicidal action over and above a dilution 1:512.

In order to reproduce conditions present during the use of antiseptics in wounds, cuts, etc., wherein large quantities of organic matter such as lymph, blood, tissue fluids, etc., are present and which impede the action of germicides, phenol coefficients were determined using Iodocholeate in the presence of much organic matter (50% horse serum) and compared with those of Tincture of Iodine.

The results of such tests are shown in Table I. Despite the presence of such a large amount of organic matter (50% horse serum) the germicidal action of Iodocholeate, as measured in terms of the phenol coefficient of its available iodine, reveals itself to be approximately three times greater than the phenol coefficient of the available iodine in the tincture.

Few antiseptics possess an action against spore-forming organisms, such as *Bacillus subtilis*. Aqueous solutions of Iodocholeate in 4% and 5% concentrations have proved themselves to be lethal to these organisms in *five minutes*. Two and five-tenths per cent solutions of this compound containing 35% alcohol killed the same organisms in a period of ten to fifteen minutes. This action is quite surprising, for further tests have shown that a 7% Tincture of Iodine did not produce an effective action even after two hours' contact, nor did a 20% Solution of Formaldehyde show any killing effect within one hour on these same spore formers.

In recent years infections by fungi, particularly those mycotic infections causing a condition known to the layman as "athlete's foot," have become increasingly prevalent.

This new compound has demonstrated highly satisfactory fungicidal properties. Lever (4) in a series of laboratory tests on common pathogenic fungi with Iodocholeate concluded that this compound had a greater killing power on fungi in the presence of organic matter than Tincture of Iodine. He also proved that the fungicidal power of this compound in Sabouraud's medium (a fungi medium) remained constant within a two-week period, whereas Tincture of Iodine became less potent (Fig. 1).

The photographs in Fig. 1 show the results of tests over a period of 10 days carried out by Deno,[1] using Iodocholeate preparations against *Trichophyton rosaceum*. The clear zone in the center of each plate shows the effective penetration and killing power of Iodocholeate on fungus growths. These results further confirm Lever's findings.

In a study to determine the reason for the germicidal and fungicidal properties of Iodocholeate, it was found, surprisingly, that these properties do not depend alone on its available iodine. For, when

[1] Asst. Professor of Biological Sciences of New Jersey College of Pharmacy, Newark, N. J.

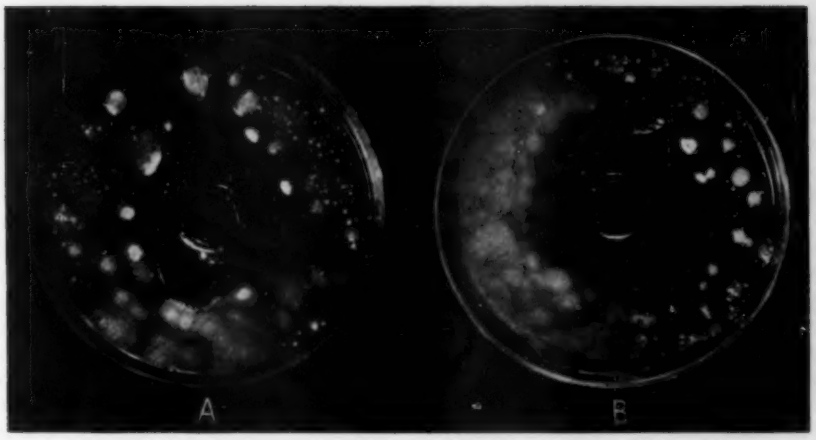


Fig. 1.—Fungicidal Activity. Organism: Trichophyton Rosaceum. (*A*) Iodocholeate Solution, (*B*) Iodocholeate Concentrated Powder.

this iodine was removed with sodium thiosulfate, the remaining compound was still effective; one part in 10.5 parts of water killed the *Staphylococcus aureus* in less than 5 minutes.

Another interesting fact about Iodocholeate is that it combines much less rapidly with organic matter than iodine and thus affords a more prolonged and better protection. Data to show this are given below. Seventy-five hundredth cc. of a 2.3% Tincture of Iodine and the same amount of an Iodocholeate solution of the same strength were each placed in five cc. of horse serum and then titrated with thiosulfate (after different time intervals) and the following was recorded:

Table II.—Rate of Combination with Organic Matter

| Time (Min.) | Tincture of Iodine (% Iodine Remaining) | Iodocholeate Solution (% Iodine Remaining) |
|---|---|---|
| 2.5 | 2.8% | 59.4% |
| 5.0 | 1.2% | 54.3% |
| 10.0 | 0.3% | 46.0% |
| 15.0 | None left | 42.1% |

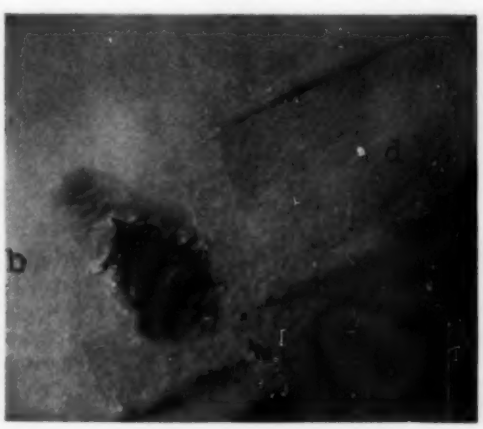


Fig. 2.—Photograph Showing Results of Patch Test on Arm. (*b*) With 5% Tincture of Iodine Ointment. (*d*) With 5% Iodocholeate Ointment.

Even though the Iodocholeate has much more available iodine than the Tincture after prolonged contact with organic matter its toxicity is considerably less. To show the low toxicity a 2.6% Iodocholeate solution was administered orally to rabbits of $4^1/_4$ lb. weight, and the results are given herewith:

Table III.—Effect of Oral Administration

Type of rabbit used: equal-sized rabbits; weight, approximately 4.5 lbs.; color, white albino

| Rabbit | Iodocholeate | Dosage | Result |
|---|---|---|---|
| 1 | 2.62% iodine | 2 cc. + 18 cc. water | No ill effects |
| 2 | 2.62% iodine | 4 cc. + 31 cc. water | No ill effects |
| 3 | 2.62% iodine | 8 cc. + 42 cc. water | Suffered loss of appetite for 4 days, then returned to normality |
| 4 | 2.62% iodine | 4 cc. undiluted | No ill effects |
| 5 | 2.62% iodine | 4 cc. undiluted | No ill effects |
| 6 | 2.62% iodine | 10 cc. undiluted | Loss of appetite for 4 days, returned to normality |
| 7 | 2.62% iodine | 10 cc. undiluted | Loss of appetite for 4 days, returned to normality |
| 8 | 2.62% iodine | 16 cc. undiluted | Loss of appetite. Death occured after 3 days |

Then, to demonstrate Iodocholeate's lack of irritation a guinea pig's back was shaved and an incision was made at the shoulder, one-half inch long and a quarter of an inch deep. Into this incision dry Iodocholeate powder (15% available iodine) was then deposited. The wound healed in four days and did not show any sign of irritation (Fig. 2).

In the close-up view of an experiment performed on an arm by Lever, shown in Fig. 2, one can readily note the large amount of necrosis caused by a Tincture of Iodine ointment, while Iodocholeate ointment shows no tissue damage.

Another important property of antiseptic solutions, according to McCulloch (5) as well as Frobisher (6), is the surface tension of these solutions. Germicides having a low surface tension penetrate into minute crevices and broken tissue more readily. Averaging a series of six surface tension results which we obtained in tests on various samples of a 2.5% Iodocholeate solution containing 35% alcohol, the surface tension was found to be 33.8 dynes at 25° C., less than half that of water, which possesses a surface tension of 73 dynes. Hence it is seen that Iodocholeate has high penetrating power.

Because of the physical and chemical properties of Iodocholeate it can be used in many ways in the field of antisepsis. Solutions of this compound have been found to be particularly suited for impregnation of gauze pads and adhesive bandages. On gauze pads, unlike inorganic iodine, Iodocholeate remains stable, non-volatile, does not destroy gauze fabrics and is neither corrosive nor irritating when applied to broken or unbroken skin (Fig. 3).

Figure 3 shows the effect of Iodocholeate ointment, prepared with an oxycholesterol-petrolatum base, and of gauze pads, impregnated with Iodocholeate solution, two of the many convenient forms in which this product can be used. The clear zones in this picture reveal the combined germicidal and penetrating action of Iodocholeate.

Also a surgical dusting or wound powder has been prepared, using bentonite, a clay powder as a base.

With bentonite, containing only 0.8% of available iodine, Iodocholeate, when tested by the F. D. A. agar cup method, demonstrates a far-reaching clear zone of penetration, whereas thymol iodide and iodoform in 100% strength fails to produce any appreciable effect.

In the final analysis the real test for a germicide lies in its clinical performance. Iodocholeate has been used clinically over a long period of time in a variety of cases.

The statement of Dr. A. H. Haskin, of the City Health Department of Newark, N. J., is typical of

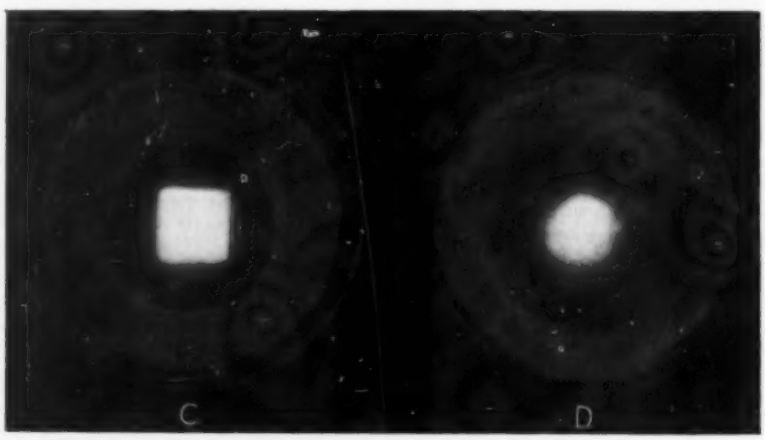


Fig. 3.—Photograph Showing Germicidal Effect of Iodocholeate Impregnated Gauze and Ointment (2.5%). Organism: Staphylococcus Aureus. (*C*) Clear Zone: 11 Millimeters. (*D*) Clear Zone: 15 Millimeters.

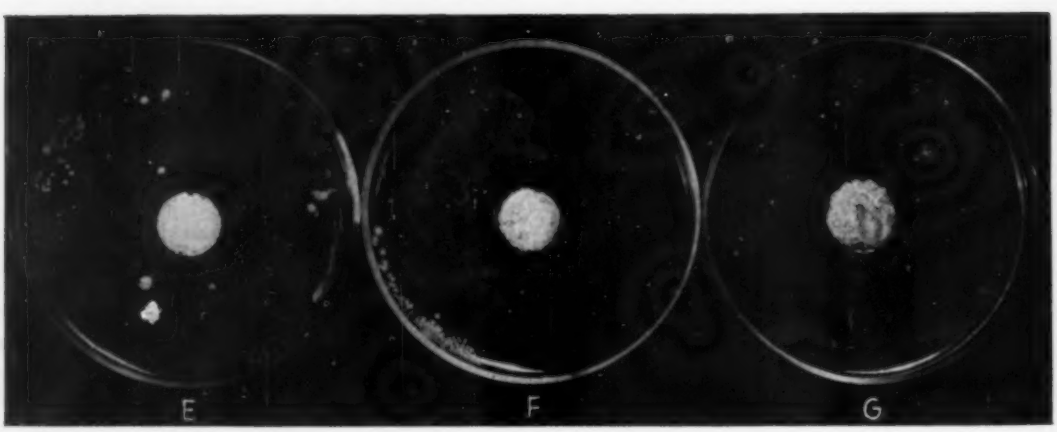


Fig. 4.—Photograph Showing Germicidal Action Compared with Iodoform and Thymol Iodide. Organism: Staphylococcus Aureus. (*E*) Iodoform (100%), (*F*) Thymol Iodide (100%), (*G*) Iodocholeate Dusting Powder (0.8%).

many reports that have been received about Iodocholeate.

Quoting him verbatim: "We have been using Solution Iodocholeate for the past two years in the surgical clinic of the Newark Health Department, and have compared its results with those of other antiseptic solutions used in this clinic. The results are gratifying.

"We have treated over 5000 cases with Iodocholeate. Among these were included ordinary lacerations, infected wounds and preoperative sterilization of the skin, and infected lesions in the mouth. In the matter of sinuses, we have placed packs soaked in Iodocholeate solution into the sinus tracts. Bandages may be used on surfaces painted with Solution of Iodocholeate without fear of necrosis.

"In antiseptic properties, we find that Iodocholeate is just as effective as Tincture of Iodine, and yet without its 'burning' properties. It also is less escharotic than Iodine and for this reason is used very effectively on lesions of the mouth, and as packs in large infected wounds. Healing of wounds occurs more quickly when Iodocholeate is used than when the U. S. P. Tincture of Iodine is used.

"It is my opinion that as an all-around antiseptic, it is superior to other solutions commonly used in this clinic."

Lever employed Iodocholeate in the treatment of mycotic infections of the skin, and found that faster improvement was obtained with Iodocholeate than with other fungicidal agents, such as sulfur-salicylic acid ointments, compound ointment of benzoic acid, N. F. and mercurochrome.

One hospital reported the use of Iodocholeate in a diversity of cases such as in appendectomy, hysterectomy, caesarian, perineorrhaphy, removal of rectal fistula and hernectomy. In all the cases in which this preparation was employed, no postoperative infection or tissue necrosis resulted.

These favorable clinical reports comprise but a few typical of many which have been received, and so confirm laboratory findings.


## SUMMARY

Further studies on Iodocholeate, the newly discovered iodine-cholеate organic germicide, have brought forth the following findings:

*Germicidal Efficiency.*—Despite the presence of organic matter (50% horse serum) which diminishes the bactericidal effectiveness of all germicides, Iodocholeate demonstrated itself to be approximately three times more effective as a bactericide against vegetative organisms than ordinary solutions of iodine under identical conditions. Against spore-forming bacteria *Bacillus subtilis* it showed a killing action within five minutes.

*Fungicidal Efficiency.*—It was proved that Iodocholeate had a greater and more prolonged fungicidal power than Tincture of Iodine in the presence of protein.

*Double Action.*—Even when the available iodine in Iodocholeate was removed, the remaining iodized cholеates demonstrated a remarkable germicidal effect.

*Oral Tolerance.*—Experiments made on rabbits show that a dose of 2 cc. of undiluted Iodocholeate per pound of body weight produces no lethal effects.

*Surface Tension.*—Tests reveal solutions of this compound to possess an exceptionally low surface tension giving it a high penetrating and diffusing power.

*Versatility.*—Because of its absence of irritation and low volatility Iodocholeate has been found to be particularly suited for impregnating gauze pads. It has also been found bactericidally effective in the form of a surgical dusting or wound powder and in ointment form.

*Clinical Evidence.*—After critical clinical observations Iodocholeate has received highly commendable reports in the field of First Aid, Surgery, Dermatology and related fields.


Further research on Iodocholeate is going to be carried on at Rutgers University, New Jersey College of Pharmacy under a fellowship grant by the Iodocholeate Products Corporation, Newark, N. J.

## REFERENCES


(1) Gershenfeld, L., and Miller, R. E., "The Bactericidal Efficiency of Iodine Solutions," JOUR. A. PH. A., 21 (1932), 894.

(2) Nye, R. N., "The Relative in Vitro Activity of Certain Antiseptics in Aqueous Solutions," *J. Am. Med. Assoc.*, 108 (1937), 280.

(3) Goedrich, P., "New Iodine-Bile Compound (Iodocholeate)," JOUR. A. PH. A., 36 (1937), 509.

(4) Lever, W. F., "Iodocholeate: A New Fungicidal Preparation: Preliminary Report," *Arch Dermat. and Syph.*, 40 (1939), 19.

(5) McCulloch, E. C., "Disinfection and Sterilization," Lea & Febiger (1936), 233.

(6) Frobisher, Martin, Jr., "Studies upon the Relationship between Surface Tension and the Action of Disinfectants, with Special Reference to Hexylresorcinol," *J. Bact.*, 13 (1927), 163.


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## NOTICE

It will be appreciated if authors will make tables for illustrations conform to the width of a single column where possible.

# A Study of Athlete's Foot and Its Control*

*J. B. Vaughan† and H. G. DeKay‡*

During the last decade or so there have come to the fore an interest in and a need for effective means of controlling certain ringworm infections. That an effective cure is yet forthcoming, there seems to be no doubt. Probably the outstanding ringworm infection, especially with respect to incidence, is the disease known popularly as athlete's foot.

Although ringworm may infect many parts of the body, statistics show the most frequent site of the infection is on the feet. The disease is almost as widespread as the common cold and as yet comparably as incurable. Nor is it confined to any particular part of the world. However, it possibly had its origin in the Eastern Archipelago (1). It seems to have been prevalent early in many of the South Sea Islands. The fact that the organisms thrive in moist, warm environment such as found on the islands mentioned, as well as sweaty shod feet of the healthy average citizen of many of the more temperate climes is a step toward explaining the whyfore of existence of this troublesome world-wide invader.

Among the reasons advanced for the spread of athlete's foot, the following seem to have special merit. First, we have to deal with the negative claim that there has been no increase in the disease. This is possible on the ground that a superior means of diagnosis exists to-day. Second, the public has been made more conscious of it through the extensive advertising of many so-called cures Third, the major and most commonly accepted reason advanced for the increased incidence of athlete's foot is the increased participation in all forms of sports which necessitates the use of common locker rooms, runways and shower baths where organisms find a ready substrate upon bare feet.

According to Osborne and Hitchcock (2), "The estimated frequency ranges from 90 per cent in male college students indulging in athletics to 50 per cent in the general adult population. In children of high school age the incidence has been estimated at about 25 to 50 per cent."

"In a recent survey of the toes of all students at the University of Pennsylvania, over 60 per cent showed ringworm infection. This was confirmed by bacteriologic methods of examination." (Goodman (3).)

The results of an investigation by Shaffer and Cary (4) in the public schools of Detroit show the incidence of epidermophytosis in school children. The general incidence of infection for girls ranged from 3 per cent of the first grade with rather regular ascendancy from grade to grade to 72 per cent of the twelfth grade girls. Among the boys, the general incidence of infection averaged 8 per cent higher than for the girls, beginning with 12 per cent and ending with 79 per cent of the twelfth grade boys.

At Purdue University the cases of athlete's foot were quite prevalent and of the 500 cases treated by the Student Health Service the first semester of 1938–1939, it was found that the majority had frequented the gymnasiums and pools of the university physical health department. In an effort to attempt to curtail the spread of this infection and with the intention of adopting the most effective means of control, this investigation was undertaken. The primary aim of this work was to make a thorough examination of sodium hypochlorite solution when used as a prophylactic measure because a careful check of the literature (2, 5, 6) revealed a number of recommendations for the use of this solution.

## EXPERIMENTAL

In order to ascertain the concentration of sodium hypochlorite solution which is adequate for prophylaxis it would be necessary to determine the actual change of the chemical content with time, the number of students using the solution and the fungicidal power at various dilutions. The task, then, was threefold:

1. Chemical analysis at intervals of time.
2. Determinations of fungicidal properties with regard to attending percentages of chemical present.
3. Count of students who used the solution.

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* An abstract of a thesis presented to the faculty of Purdue University in partial fulfilment of the requirements for the degree of Master of Science by J. B. Vaughan.

† Assistant in Pharmaceutical Chemistry, Purdue University.

‡ Associate Professor of Pharmacy, Purdue University.

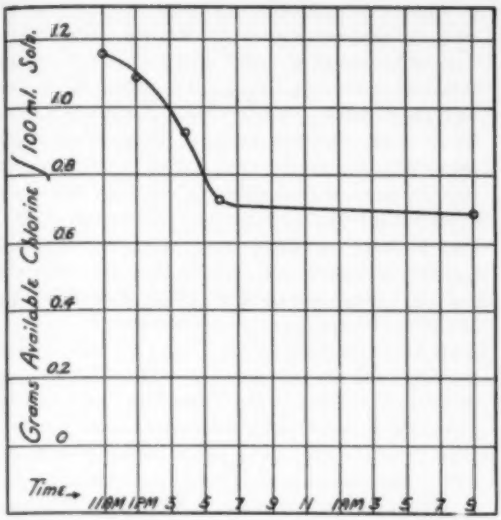


Fig. 1.—Curve No. I Showing Changes in Content of Available Chlorine in Sodium Hypochlorite Foot Bath.

With the coöperation of the athletic department, foot baths were placed in doorways leading to the general and varsity shower-rooms in the Fieldhouse. The containers were three feet square, with a capacity of about twenty-five gallons. Thirteen gallons of sodium hypochlorite solution calculated to be approximately one per cent of available chlorine were placed in these pans daily. Samples were collected at hourly intervals and analyzed for available chlorine. This was repeated several times a week over a three-month period.

The method of analysis used is that found in the U. S. P. XI Solution of Sodium Hypochlorite; ten-ml. samples instead of weighed samples were determined.

Graphs were made in which Gm. available chlorine per 100-ml. sample is plotted against time in hours. Figure 1 and Figure 2, Curves III, IV and V are typical examples of the changes in chlorine content which took place during the day. In general there resulted a loss of chlorine content with the lapse of time. This loss is not to be attributed to the escape of chlorine gas from the solution. During the hours when more students made use of the foot baths, loss of chlorine content was greater. This is due, then, either to the bodily removal of the chemical or possibly to dilution. The latter contention is not plausible in view of the fact that observation showed the baths decreased in volume.

As contrasted with the decrease in chlorine content between the hours of four and seven (time when large numbers of students made use of the showers), the loss during the entire night (when no students used the showers) shows very little change; therefore it seems evident that the diminution in available chlorine by evaporation is more or less unimportant. See Fig. 1.

As shown in Fig. 2, Curve II, the foot bath was made to contain approximately 0.5 per cent available chlorine. The percentage of chlorine, after the day's use, dropped to 0.23. Although it was not possible to keep a complete record of the number of students using the foot baths daily, observation at specific times showed the percentage decrease was proportional to the number of users.

A slight rise in some of the curves shortly after introduction of the bath and other slight deviations may be attributed to failure in securing representative samples.

These foot baths accommodated an average of 400 students per day; during that period, the available chlorine decreased from one per cent to four-tenths of one per cent. As will be shown later, even this lower concentration possesses high fungicidal power.

Figure 2, Curve III, shows 0.97 per cent available chlorine at 9:00 A.M., 0.90 per cent at 11:00 A.M., 0.86 per cent at 2:00 P.M. Then the curve begins a rather steep descent until at 6:00 P.M. it approaches the 0.5 per cent mark. At the close of the day, at 9:00 P.M., the chlorine content has fallen to 0.45 per cent, or a loss during the twelve hours of 0.52 per cent.

In order to carry on the work of examination of chemical substances for fungicidal properties, the successful culturing of representative fungi, or at least a representative fungus, is obviously necessary. In this study *Trichophyton rosaceum* (common in athlete's foot) is the organism employed in all cases. The fact that *Trichophyton rosaceum* has conspicuous macroscopic characteristics makes it the organism of selection. It has also a reasonably high resistance to action of fungicides.

Of course it is recognized that although a substance proves completely fungicidal toward the organism, *T. rosaceum*, other fungi of athlete's foot

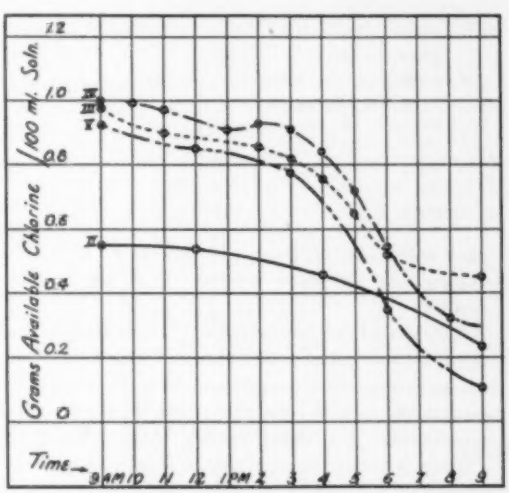


Fig. 2.—Curves Nos. II–V Showing Changes in Content of Available Chlorine in Sodium Hypochlorite Foot Bath.

Table I.—Fungicidal Action of Sodium Hypochlorite

| Tube No. | Seconds Exposed | Growth After 18 Hrs. | 1 Day | 2 Days | 5 Days | 10 Days |
|---|---|---|---|---|---|---|
| A. 0.1123 Gm. Cl/100 ml. | | | | | | |
| 77 | 10 | − | − | + | + | + |
| 78 | 20 | − | − | − | − | − |
| 79 | 30 | − | − | − | − | − |
| 80 | 60 | − | − | − | − | − |
| Control | | + | + | + | + | + |
| B. 0.2154 Gm. Cl/100 ml. | | | | | | |
| 97 | 10 | − | − | − | + | + |
| 98 | 20 | − | − | − | − | − |
| 99 | 30 | − | − | − | − | − |
| 100 | 60 | − | − | − | − | − |
| Control | | + | + | + | + | + |
| C. 0.2951 Gm. Cl/100 ml. | | | | | | |
| 89 | 10 | − | − | − | − | − |
| 90 | 20 | − | − | − | − | − |
| 91 | 30 | − | − | − | − | − |
| 92 | 60 | − | − | − | − | − |
| Control | | + | + | + | + | + |
| D. 0.4127 Gm. Cl/100 ml. | | | | | | |
| 81 | 10 | − | − | − | − | − |
| 82 | 20 | − | − | − | − | − |
| 83 | 30 | − | − | − | − | − |
| 84 | 60 | − | − | − | − | − |
| Control | | + | + | + | + | + |
| E. 0.5038 Gm. Cl/100 ml. | | | | | | |
| 65 | 10 | − | − | − | − | − |
| 66 | 20 | − | − | − | − | − |
| 67 | 30 | − | − | − | − | − |
| 68 | 60 | − | − | − | − | − |
| Control | | + | + | + | + | + |
| F. 0.6197 Gm. Cl/100 ml. | | | | | | |
| 73 | 10 | − | − | − | − | − |
| 74 | 20 | − | − | − | − | − |
| 75 | 30 | − | − | − | − | − |
| 76 | 60 | − | − | − | − | − |
| Control | | + | + | + | + | + |
| G. 0.7163 Gm. Cl/100 ml. | | | | | | |
| 93 | 10 | − | − | − | − | − |
| 94 | 20 | − | − | − | − | − |
| 95 | 30 | − | − | − | − | − |
| 96 | 60 | − | − | − | − | − |
| Control | | + | + | + | + | + |
| H. 0.8756 Gm. Cl/100 ml. | | | | | | |
| 61 | 10 | − | − | − | − | − |
| 62 | 20 | − | − | − | ± | ± |
| 63 | 30 | − | − | − | − | − |
| 64 | 60 | − | − | − | − | − |
| Control | | + | + | + | + | + |
| I. 0.9512 Gm. Cl/100 ml. | | | | | | |
| 69 | 10 | − | − | − | − | − |
| 70 | 20 | − | − | − | − | − |
| 71 | 30 | − | − | − | − | − |
| 72 | 60 | − | − | − | − | − |
| Control | | + | + | + | + | + |

may react contrariwise. However, *T. rosaceum* is typical and certainly worthy of giving indicative results.

Sabouraud culture medium (7) was prepared and used as the culture medium. A stock culture of the organism was transferred to a Petri dish containing the medium and allowed to incubate at room temperature for three weeks. After this period, growth covered the agar plates. An inoculum consisting of the organisms from the culture plate in normal saline was next prepared.

Samples of the foot bath collected previously were redetermined and the prepared inoculum subjected to whatever fungicidal action there might be with various concentrations of sodium hypochlorite. The accompanying table shows different strength solutions of sodium hypochlorite selected. In each case the organisms were exposed for 10, 20, 30 and 60 seconds. Growth positive or negative is indicated after 18 hours, 1 day, 2, 5 and 10 days incubation at room temperature. Negative tubes were observed for two-week periods. Divisions A, B, C, etc., indicate ascending concentrations of chlorine content beginning with 0.1123 Gm. available chlorine per 100 ml. sample and ending with 0.9512 Gm. available chlorine per 100 ml. sample.

In A and B, Tubes Nos. 77 and 97 show positive growth with 10 seconds exposure. With longer periods of exposure even these low concentrations (0.1123% and 0.2154%) show no growth.

In D, Tube No. 81 shows a slight growth after 5 days incubation as indicated by the symbol, ±.

In H, Tube No. 62 shows a slight positive growth. This is undoubtedly due to contamination through some mishap in technique, as Tube No. 61, which is the 10-second-exposed tube of the series, is negative.

C, E, F, G and I all show negative growth with 10, 20, 30 and 60 seconds exposure.

An examination of Table I, then, reveals that in all strengths of sodium hypochlorite tried (0.1123% to 0.9512%) positive growth is inhibited after the organisms are exposed for twenty seconds.

Other chemicals examined mycologically included: mercuric chloride, copper sulfate, zinc chloride, alum, sulfosalicylic acid, sodium chloride, sodium thiosulfate and formaldehyde.

Mercuric chloride, copper sulfate, zinc chloride and ammonium alum in 1:1000 dilutions were found to be non-fungicidal to *T. rosaceum*.

Sodium chloride in ten per cent solution, sodium thiosulfate in ten per cent solution and sulfosalicylic acid in one per cent solution proved to be non-fungicidal to *T. rosaceum* with as long as sixty seconds exposure.

Five per cent solution of formaldehyde inhibits growth of *T. rosaceum* with twenty seconds exposure.

## SUMMARY

In summarizing, it may be said that a foot bath containing thirteen gallons of one per cent (available chlorine) sodium hypochlorite solution is very satisfactory as a prophylactic to athlete's foot for daily use of four hundred persons. This conclusion is further supported by the clinical finding of Dr. S. J. Miller, Director of the Student Health Service at Purdue University, who reports a decrease in the number of new cases of athlete's foot since the installation of sodium hypochlorite foot baths in the Fieldhouse.

REFERENCES

(1) Levine, D. D., *Hygeia*, 14 (1936), 728.

(2) Osborne, E. D., and Hitchcock, B. S., *J. Am. Med. Assoc.*, 97 (1931), 453.

(3) Goodman, H., "Treatment of Common Skin Diseases," Medical Lay Press (1932).

(4) Shaffer, L. W., and Cary, W. H., Jr., *J. Mich. State Med. Soc.*, 32 (1933), 648.

(5) Osborne, E. D., Putman, E. D., and Rickloff, R. J., *N. Y. State J. Med.*, 33 (1933), 1270.

(6) Circular, "Control of Ringworm in Gymnasia," *Bull. Indiana State Board of Health* (Jan. 10, 1938).

(7) Gershenfeld, L., "Bacteriology and Sanitary Science," Lea & Febiger, Philadelphia (1929).

# A Method for the Determination of Peptic Activity*

*By C. J. Klemme† and Lee Worrell‡*

## INTRODUCTION

This study is the result of an attempt to find in the literature a relatively short, convenient, accurate method of assay for preparations containing pepsin, which could be used for routine assays in a study of the stability of such preparations.

Although numerous assay methods for pepsin have been proposed, original references to many of which may be found in publications by Sherman and Neun (1), Vahlteich and Glover (2), Northrop (3), Greenberg (4), Jenkins and Hoshall (5) and Waksman and Davison (6), none have proved entirely satisfactory. The variability of the substrate and correlation between the actual measurement of the amount of digestion and the concentration of enzyme causing the digestion have been outstanding difficulties involved.

## EXPERIMENTAL

Preliminary experiments, in which several of the above-mentioned methods were tested, indicated that the method of Jenkins and Hoshall (5) offered the most convenient means of determining the amount of digestion. It was necessary to make several important changes, however, before accurate results could be obtained.

*Method.*—Using a solution 10 cc. of which contain 0.05 Gm. of reference pepsin, determine the $K$ value of the lot of casein to be used in the assays by the method described below. The reference pepsin is preferably a sample of the pepsin used in the preparations to be assayed; if this is not available, the "Reference Pepsin" of the U. S. P. XI may be used.

To about 200 cc. of 0.0800$N$ hydrochloric acid contained in a one-liter Erlenmeyer flask, add 17.2 Gm. of casein (according to Hammarsten). Shake the flask until the casein is thoroughly moistened and evenly dispersed. Add enough 0.0800$N$ hydrochloric acid to make the total volume of acid exactly 400 cc. Stopper the flask loosely and place in a bath of boiling water for exactly 30 minutes.[1] Remove and quickly cool (under the tap) to room temperature. The product should contain no undissolved particles of casein. Pipette 70 cc. of this substrate into a 125-cc. Erlenmeyer flask. Stopper the flask loosely and place it in a constant temperature bath, previously regulated to maintain a temperature of 55° C., in such a position that the neck of the flask is above the water in the bath. After the flask has remained in the bath exactly 10 minutes, and without removing it from the bath, add by means of a pipette 10 cc. of a dilution of the preparation to be assayed and mix by gently shaking. The preparation is diluted with distilled water so that 10 cc. of the dilution contain 0.02 Gm. to 0.08 Gm. of pepsin. Preliminary trial assays may be necessary in order to determine the proper dilution. Allow the flask, loosely stoppered, to remain in the bath exactly 30 minutes after the sample is added. Remove from the bath and immediately add exactly 20 cc. of a solution of sodium sulfate (20 Gm. of the anhydrous salt in 100 cc. of distilled water), mix well and cool under the tap to 25° C. Filter through hardened filter paper. Titrate a 25-cc. aliquot portion of the clear filtrate with $N$/10 sodium hydroxide, using phenolphthalein as the indicator. Run a blank, using 10 cc. of distilled water instead of the pepsin solution, in exactly the same manner. At the end-point of the blank titration, add 2.50 cc. of the dilution used in the determination, and complete the titration. The difference in acidity between the determination and blank, expressed as cc. of $N$/10 acid, is an index of the relative proteolytic activity and is designated in the calculations as $X$.

Calculations: To determine the $K$ value of the casein, substitute the value obtained for $X$ in the reference determination into the equation

$$K = X/2.24$$

To determine the amount of active pepsin in the preparation assayed, substitute the value obtained for $X$ in the assay into the equation

$$\sqrt{E} = X/K$$

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* An abstract of a thesis submitted to the faculty of Purdue University in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

† Professor of Pharmaceutical Chemistry, Purdue University, School of Pharmacy.

‡ Eli Lilly & Co. Fellow, Purdue University, School of Pharmacy.

[1] In high altitudes where the boiling point of water is appreciably below 100° C., the time required for solution may be longer. This time would have to be determined experimentally.

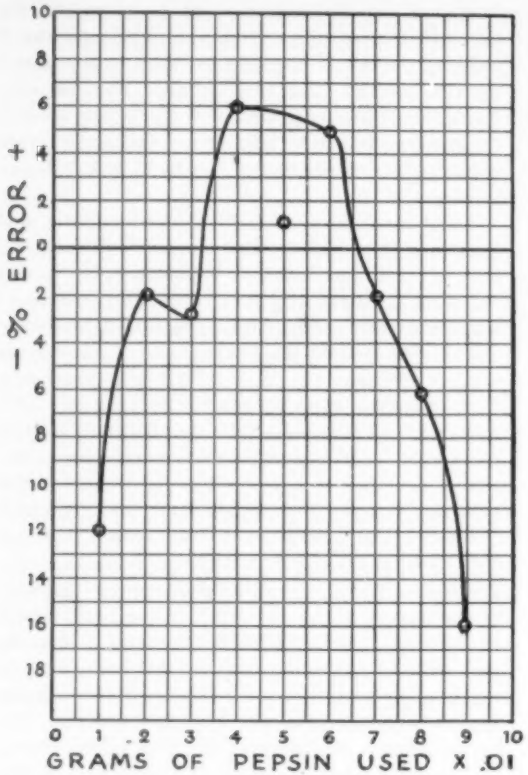


Fig. 1.—Graph Showing the Amount Digested in Relation to Pepsin Concentration.

Solve for $E$, which is the relative concentration of pepsin. Divide $E$ by 100 to obtain the fraction of a Gm. of pepsin in the 10 cc. of the dilution used. Multiply by the appropriate factor, according to the dilution used, to obtain the concentration of pepsin in the preparation assayed.

The entire method of preparation of the substrate has been changed to eliminate errors found to be due to variations between the individual substrates of the older method. Experiment showed that it is necessary to heat the mixture at least 30 minutes before a homogenous substrate is produced. Longer periods of heating increase slightly the amount of digestion subsequently obtained, but the increases are not sufficient to compensate for the extra time involved. It was found that the use of 0.0800$N$ hydrochloric acid provides a substrate of the optimum $p_H$ for peptic digestion under the conditions of the method. Substrates prepared in the manner described give reproducible results as long as the same lot of casein is used; when different lots of casein are compared, slight variations occur in the results. Hence it is necessary to standardize each lot of casein used against a reference pepsin.

*Experiments to Determine Optimum Digestion Temperature and Time.*—The results of experiments to determine the optimum time and temperature for digestion indicate that digestion for 30 minutes at 55° C. is most suitable.

The preliminary experiments showed that within a certain range of concentrations of pepsin the Schütz Law is valid, *i. e.*, the amount of digestion is proportional to the square root of the amount of enzyme used. It was necessary to determine the exact range of pepsin concentrations in which this proportionality held true. The results tabulated below indicate that the calculated results check the known amounts of pepsin used within 6 per cent, when the range of pepsin concentration is from 0.02 Gm. to 0.08 Gm. This is more clearly shown in the accompanying graph. The abnormal value obtained at 0.05 Gm. is probably due to the end-point error.

Table I.—Results of Digestion Experiments

Casein Lot 79080. $K = 2.10$

| Gm. Pepsin Used | Diff. in Acidity Cc. 0.1$N$ HCl | Gm. Pepsin Found | Per Cent Error |
|---|---|---|---|
| 0.0900 | 5.78 | 0.0756 | −16.0 |
| 0.0800 | 5.75 | 0.0751 | − 6.1 |
| 0.0700 | 5.50 | 0.0686 | − 2.0 |
| 0.0600 | 5.27 | 0.0630 | + 5.0 |
| 0.0500 | 4.73 | 0.0506 | + 1.2 |
| 0.0400 | 4.32 | 0.0424 | + 6.0 |
| 0.0300 | 3.59 | 0.0292 | − 2.7 |
| 0.0200 | 2.94 | 0.0196 | − 2.0 |
| 0.0100 | 1.98 | 0.0088 | −12.0 |

Twelve different samples of pepsin and thirty-eight different samples of liquid preparations containing pepsin have been assayed by this method. Six assays were run on each of the liquid preparations, three each on two different dilutions and four assays were run on each sample of pepsin. In every case checking results were obtained. Most of these samples were purchased from retail drug stores.

## CONCLUSIONS

1. A convenient method of assay for peptic activity, especially suited for stability studies, has been devised using the method of Jenkins and Hoshall (5) as a starting point.

2. An investigation of the peptic activity of liquid pepsin preparations purchased from various retail stores indicates that the majority of liquid pepsin preparations so purchased are above the N. F. VI standard for peptic activity, but none of them have activity equivalent to the amount of pepsin added at the time of preparation.

## REFERENCES

(1) Sherman, H. C., and Neun, D. E., *J. Am. Chem. Soc.*, 38 (1916), 2199.

(2) Vahlteich, H. W., and Glover, C. C., JOUR. A. PH. A., 10 (1921), 595.

(3) Northrop, J. H., *J. Gen. Physiol.*, 16 (1932), 41.

(4) Greenberg, H. L., JOUR. A. PH. A., 20 (1931), 1032.
(5) Jenkins, G. L., and Hoshall, E. M., *Ibid.*, 22 (1933), 625.
(6) Waksman and Davison, "Enzymes" (1926), Williams and Wilkins Co.

# A Study of the Assay of Ginger*

*By Robert Tzucker† and C. B. Jordan‡*

## INTRODUCTION

Ginger has been known and used in medicine for a great many years. Although the use of ginger in medicine has fallen off to a great extent, it is still widely employed as a spice and in the preparation of various beverages.

The method of assay for ginger has never been a satisfactory one. The establishment of an accurate assay method is difficult due to the resinous consistency of the active constituents. The Ninth Revision of the United States Pharmacopœia was the first revision to include a standard for ginger. In this revision, the water-soluble extract, the non-volatile ether-soluble extract and the alcohol-soluble extract were determined. The Tenth Revision determined the water-soluble extract and the non-volatile ether-soluble extract. The Eleventh Revision determines the amount of ether-soluble extract, assuming, and rightly so, that the active or pungent principle is ether-soluble.

The method of the Eleventh Revision consists of extracting a portion of the drug with ether and weighing the residue after volatilization of the ether. Water-bath temperature is used to volatilize the ether. The objections to this method of assay are:

1. Individuals vary in their olfactory sensitivity to ether. Some operators may be unable to detect ether odor even though ether still remains in the extract; this, of course, will introduce an error in weight.

2. The active volatile principles may be partially but not entirely removed by heating at water-bath temperature, and therefore, the residue may not be a measure of all of the volatile constituents. Since the amount of volatile material coming off may vary, the weight of the residue will vary with different samples.

This study was carried out in an attempt to find a more satisfactory assay method.

## HISTORICAL REVIEW OF THE METHODS OF ANALYSIS FOR GINGER

A method for the standardization of ginger was not introduced until the Ninth Revision of the United States Pharmacopœia. In that Revision the water-soluble extract, the non-volatile ether-soluble extract and the alcohol-soluble extract were determined.

Garnett and Grier (1909) used a shake-out method to determine the amount of gingerol, which has been shown to be the active principle. The method consisted of extracting the drug with ether, volatilizing the ether and boiling the residue with repeated portions of petroleum spirit. The petroleum ether is shaken out with successive portions of sixty per cent alcohol, leaving the volatile oil, fatty oil and much coloring matter in the petroleum spirit. The alcoholic solution is then washed with further portions of petroleum spirit to remove the last traces of fat. The alcohol is recovered or volatilized and the residual liquid shaken out with three successive portions of ether. The ether is then volatilized and the gingerol is dried to constant weight and weighed. Garnett and Grier (1907) claim that capsicum is a common adulterant of ginger. They give a method for determining the presence of capsicum. It consists of digesting on a water-bath about ten cc. of the extract with a small quantity of caustic alkali for fifteen minutes. The solvent is volatilized and the residue is acidified slightly with hydrochloric acid. It is then shaken out with a small portion of ether and the ethereal solution is tasted. In the case of pure ginger, the pungency will be found to have entirely disappeared, while if capsicum be present, the pungent, biting taste is at once recognized. One part of capsicum in one hundred parts of ginger may be detected in this manner.

Ginger extracts also give a specific color reaction. This test is carried out as follows:

Dilute 10 cc. of the extract to 30 cc.; evaporate to 20 cc. and extract this solution with 20 cc. of ether. Allow the ether to evaporate spontaneously in an evaporating dish and add 5 cc. of seventy-five per cent sulfuric acid and 5 mg. of vanillin to the residue. Allow this mixture to stand for about fifteen minutes and then add an equal volume of water. An azure-blue color develops in the presence of ginger extracts.

* An abstract of a thesis submitted to faculty of Purdue University in partial fulfilment of the requirements for the Master of Science degree, June 1938. The thesis contains a bibliography of fifty references.

† Assistant in Pharmaceutical Chemistry, Purdue University, 1937–1938.
‡ Dean, Purdue University, School of Pharmacy.

Clevenger (1928) claims that the method of the Ninth and Tenth Revisions of the United States Pharmacopœia of determining the volatile ether-soluble extractive is empirical and subject to several criticisms. These are:

1. The loss in weight does not always represent the volatile oil which usually is the desirable or active constituent; it may include volatile materials resulting from the decomposition of unknown substances.

2. The prolonged heating at 100° C. of the residue, which is necessary to remove the volatile matter completely from the ether-soluble extractive, increases the possibility of decomposition with subsequent erroneous results.

3. The volatile constituents of the product in question are not available for subsequent examination which is desirable in many instances.

Clevenger (1928) has devised an apparatus for the determination of volatile oils by means of which the amount of the volatile oil can be read directly. He has worked out standards for ginger and its preparations but the range of these standards seems rather wide. These are:

1. Volatile oil, 1.2 to 3.0 per cent.

2. Specific gravity of the volatile oil, 0.876 to 0.885.

3. Optical rotation of the volatile oil (25° C.) −40° to −56°.

4. Refractive index of the volatile oil (20° C.), 1.490 to 1.493.

5. Non-volatile ether-soluble extractive 3.5 to 7.1 per cent.

6. Iodine value of the non-volatile ether-soluble extractive 36 to 50.

7. Saponification value of the non-volatile ether-soluble extractive 45 to 70.

Clevenger claims that although the physical constants of the extractives vary considerably, the addition of appreciable amounts of an adulterant may be readily detected by deviations in the above mentioned constants.

## EXPERIMENTAL PART

*I. U. S. P. XI Method of Assay for Ginger.*—In order to determine the accuracy of the U. S. P. XI method for the assay of ginger, several samples were analyzed by the official method. This consists of extracting a weighed portion of the drug, twenty Gm., with ether in a Soxhlet extraction apparatus for six hours. The liquid is then evaporated on a water-bath until the odor of ether is no longer perceptible. The container holding the extractive is then placed in a desiccator for twelve hours and weighed. The weight of the extract should be not less than nine-tenths of a Gm. or 4.5 per cent of the weight of the sample. This experiment was carried out on a sample of Jamaica ginger which had been stored in paper cartons in the basement for a period of eight months. Table I shows the results of this experiment.

Table I.—Ether Extractive

Twenty Gm. of Sample Used for Each Extraction

| Sample Number | Per Cent of Residue | Weight of Residue |
|---|---|---|
| 1 | 3.94 | 0.7870 |
| 2 | 3.94 | 0.7871 |
| 3 | 4.46 | 0.8932 |
| 4 | 4.56 | 0.9119 |
| 5 | 4.15 | 0.8299 |
| 6 | 4.22 | 0.8439 |
| 7 | 4.19 | 0.8389 |
| 8 | 4.21 | 0.8411 |
| 9 | 3.89 | 0.7786 |
| 10 | 3.93 | 0.7862 |
| 11 | 3.96 | 0.7913 |
| 12 | 3.95 | 0.7907 |
| 13 | 4.28 | 0.8560 |
| 14 | 4.28 | 0.8555 |
| 15 | 4.26 | 0.8510 |
| 16 | 4.26 | 0.8523 |

From this experiment, the fact is evident that this sample of ginger is not of Pharmacopœial strength and that concordant results are difficult to obtain by this assay method. A possible reason for the low results may be the loss of volatile material under the conditions of storage.

*II. Effect of Water-Bath Temperature on the Ether-Soluble Extractive.*—The next experiment was carried out to determine the effect of water-bath temperature on the residue over different periods of time. Since the sample of ginger used in the first experiment was not of U. S. P. strength, a fresh sample stored in an air-tight container was obtained. This sample of ginger was used in all of the remaining experiments. Table II shows the results of this experiment.

Table II.—Effect of Heating on Yield of Extractive

| | Per Cent of Residue | | | | | | |
|---|---|---|---|---|---|---|---|
| | | After Heating on Water-Bath, Time in Hrs. | | | | | |
| Sample Number | After Spontaneous Evaporation of the Ether | 1 | 2 | 3 | 4 | 5 | 6 |
| 1 | 4.92 | 4.55 | 4.32 | 4.22 | 4.15 | 4.10 | 4.02 |
| 2 | 4.88 | 4.53 | 4.18 | 4.09 | 4.03 | 3.96 | 3.88 |
| 3 | 4.87 | 4.50 | 4.12 | 4.01 | 3.90 | 3.84 | 3.79 |
| 4 | 4.86 | 4.43 | 4.19 | 4.11 | 4.00 | 3.93 | 3.88 |
| 5 | 5.02 | 4.54 | 4.26 | 4.16 | 4.02 | 3.94 | 3.86 |
| 6 | 4.98 | 4.62 | 4.34 | 4.21 | 4.07 | 4.01 | 3.92 |
| 7 | 4.90 | 4.58 | 4.21 | 4.13 | 3.96 | 3.87 | 3.80 |
| 8 | 4.87 | 4.41 | 4.22 | 4.10 | 3.98 | 3.84 | 3.76 |
| 9 | 5.21 | 4.62 | 4.50 | 4.37 | 4.31 | 4.26 | 4.19 |
| 10 | 5.10 | 4.56 | 4.39 | 4.22 | 4.12 | 4.05 | 3.98 |

By this experiment the fact is brought out that the length of time that the residue is exposed to water-bath temperature will greatly affect the weight of the residue. Two hours exposure changed all of the samples except one from U. S. P. strength to below the U. S. P. standard. This bears out the point previously mentioned, that the sensitivity of the individual in being able to detect the presence of ether will markedly affect the amount of residue obtained.

*III. Volatility of the Ether-Soluble Extractive.*—The next experiment was carried out to determine the volatility of the ether-soluble extract in air. Table III shows the results.

Table III.—Loss in Weight of Extract on Exposure to Air

| Sample Number | Per Cent of Residue: After Spontaneous Evaporation of the Ether | After Exposure to Air, Time in Hrs. 5 | 12 | 36 | 72 |
|---|---|---|---|---|---|
| 1 | 4.73 | 4.70 | 4.68 | 4.54 | 4.52 |
| 2 | 4.74 | 4.72 | 4.69 | 4.66 | 4.64 |
| 3 | 4.79 | 4.77 | 4.75 | 4.72 | 4.70 |
| 4 | 4.77 | 4.74 | 4.72 | 4.70 | 4.67 |

The volatile constituents, as shown by this experiment, will volatilize to some extent at room temperature.

*IV. Determination of the Non-Volatile Ether-Soluble Extractive.*—Since the active constituent is reported as being non-volatile and ether-soluble, the next experiment was performed to determine the non-volatile ether-soluble extractive. The drug was extracted with ether as in the U. S. P. method and the ether permitted to evaporate spontaneously. An attempt was then made to dry the remaining residue to constant weight at 100° C., but, as Table IV shows, this was almost impossible because the residue lost weight indefinitely.

Table IV. Non-Volatile Ether Extractive

Twenty Gm. of Sample Used for Each Extraction

| Sample Number | Per Cent of Residue: 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| After spontaneous evaporation of ether | 5.02 | 4.98 | 5.00 | 4.88 |
| In oven 60° C., 1 hour | 4.81 | 4.77 | 4.84 | 4.65 |
| In oven 100° C., 17 hours | 3.66 | 3.60 | 3.69 | 3.57 |
| In oven 100° C., 35 hours | 3.50 | 3.44 | 3.51 | 3.34 |
| In oven 100° C., 53 hours | 3.42 | 3.34 | 3.41 | 3.25 |
| In oven 100° C., 83 hours | 3.30 | 3.25 | 3.32 | 3.10 |
| In oven 100° C., 119 hours | 3.23 | 3.18 | 3.24 | 3.02 |
| In oven 100° C., 191 hours | 3.05 | 3.02 | 3.02 | 2.85 |

The residue was tasted after 191 hours and there was no pungency present, showing either that the pungent principle is volatile at 100° C., or that it is oxidized to some non-pungent form.

*V. Wirth's Method for the Determination of the Amount of Volatile Constituents.*—E. H. Wirth of the University of Illinois College of Pharmacy, in a paper reported at the 1937 meeting of the American Association for the Advancement of Science, presented the following method for the determination of the total volatile material in ginger: He determined the moisture content by the toluene method. Then he determined the total volatile material by the oven method (dried the drug at 100° C. until there was no loss in weight). By subtracting the amount of moisture from the total volatile material, a value for the volatile material was obtained.

An attempt was made to follow this procedure. The amount of moisture was determined by the toluene method and was found to be 6.05 per cent. To determine the total volatile material the drug was weighed in small beakers and thus exposed to oven heat at 100° C. It was very difficult to weigh the drug as it absorbed moisture so rapidly that it was almost impossible to make an accurate weighing. The weighings were, therefore, made very rapidly but the results did not check. Glass stoppered weighing bottles were then substituted for the beakers. The drug was weighed and exposed for one hour periods in the oven. The results are shown in Table V.

Table V.—Volatile Extractive by Wirth's Method

| Sample Number | Per Cent of Volatile Material: 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Dried 1 hour at 100° C. | 2.74 | 3.51 | 3.09 | 3.51 | 3.22 | 3.28 |
| Dried 2 hours at 100° C. | 5.32 | 5.56 | 5.09 | 5.27 | 4.87 | 4.96 |
| Dried 3 hours at 100° C. | 6.11 | 6.37 | 5.74 | 6.12 | 5.81 | 6.08 |
| Dried 4 hours at 100° C. | 4.24 | 6.48 | 5.94 | 6.28 | 6.04 | 6.22 |
| Dried 5 hours at 100° C. | 6.46 | 6.72 | 6.39 | 6.52 | 6.32 | 6.63 |
| Dried 6 hours at 100° C. | 6.71 | 7.06 | 6.64 | 6.73 | 6.61 | 6.94 |
| Dried 7 hours at 100° C. | 6.99 | 7.15 | 6.93 | 6.90 | 6.79 | 7.11 |
| Dried 8 hours at 100° C. | 7.07 | 7.19 | 6.98 | 7.01 | 6.92 | 7.17 |
| Dried 9 hours at 100° C. | 7.12 | 7.25 | 7.05 | 7.14 | 7.02 | 7.22 |
| Dried 10 hours at 100° C. | 7.20 | 7.33 | 7.11 | 7.27 | 7.12 | 7.30 |
| Dried 11 hours at 100° C. | 7.26 | 7.39 | 7.21 | 7.34 | 7.23 | 7.41 |
| Dried 347 hours at 100° C. | 9.26 | 9.39 | 9.33 | 9.19 | 9.34 | 9.46 |

Table V shows that it is almost impossible to dry the drug to constant weight; this seems to contradict Wirth's conclusions. Decomposition seems to take place and the drug becomes dark in color. It also loses its pungent taste. This drug was extracted in a Soxhlet extraction apparatus with ether for six hours but only a very small amount of extractive was obtained. The pungent principle apparently is volatilized or oxidized by heat. Since the drug could not be satisfactorily dried to constant weight, this line of experimentation was discontinued.

*VI. Shake-Out Method for the Determination of Gingerol.*—Garnett and Grier (1909) used a shake-out method to determine the amount of gingerol which has been said to be the chief constituent of ginger. They merely remove the fat and other materials from the ether extract by the use of various solvents. Gingerol has been shown to be somewhat soluble in these solvents and the result would not be an accurate measure of the quantity of gingerol. The procedure proved unsatisfactory in the hands of the operator because of the difficulty of separating the solvents used. Since this method was so closely related to that employed in the determination of the ether-soluble extract, with certain modifications, it was thought useless to pursue this line of investigation.

*VII. Qualitative Test for Ginger Extracts.*—Ten cubic centimeters of extract were diluted to 30 cc. This solution was evaporated to 20 cc. and then extracted with 20 cc. of ether. The ether was permitted to evaporate spontaneously in an evaporating dish and 5 cc. of a 75% solution of sulfuric acid and 5 mg. of vanillin were added to this residue. This mixture was permitted to stand for 15 minutes and then an equal volume of water was added. An azure-blue color appeared. This test is of value only in the testing for the complete absence of ginger extracts. If any amount of ginger extract is present, this blue color will be formed.

*VIII. Clevenger's Method for the Determination of the Volatile Oil.*—Clevenger (1928) devised an apparatus for the determination of the amount of

volatile oil in a drug. This apparatus was used and fairly good checks were obtained. Table VI shows the results.

Table VI.—Yield of Volatile Oil

| Sample Number | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Per cent of volatile oil | 2.38 | 2.24 | 2.36 | 2.54 |

Apparatus for the determination of the other constants for ginger extracts given by Clevenger was of a special kind and could not be obtained. Since the procedure was quite long and involved, and could not be practically incorporated into the Pharmacopœia, this line of investigation was discontinued.

*IX. New Method for the Determination of the Ether-Soluble Extractive.*—In the U. S. P. XI the ether-soluble extractive is determined by weighing the residue from the ether extract after volatilization of the solvent. If a satisfactory method could be devised involving the weighing of the marc after ether extraction, more accurate results could probably be obtained. The next line of investigation was conducted with this end in view.

A paper extraction thimble was extracted with ether in a Soxhlet extraction apparatus for two hours; the ether was volatilized and the thimble weighed. The drug was then placed in the thimble and extracted with ether for six hours. The ether was volatilized from the extracted drug and thimble, and the thimble and extracted drug were then weighed. The loss in weight of the drug represented the ether-soluble extractive. Oven heat at 60° C. was used to volatilize the ether.

In the first attempts with this experiment, the thimble was weighed in air. This was quite difficult because the thimble absorbed moisture so rapidly that an accurate weighing could not be made. Next an attempt was made to use glass extraction thimbles but they proved to be unsatisfactory, so the use of paper extraction thimbles was again resorted to.

The next attempt involved the use of special glass-stoppered weighing bottles into which the extraction thimbles fit and thereby insure accurate weighings. The paper thimble was extracted with ether for two hours in a Soxhlet extraction apparatus; the ether was volatilized by the use of oven heat at 100° C., and the thimble was weighed in the closed weighing bottle. The drug, previously dried in a sulfuric acid desiccator for 48 hours, was then placed in the thimble and extracted with ether for six hours. The ether was volatilized and the thimble and drug weighed. The loss in weight of the drug represented the ether-soluble extractive. Table 7 shows the results obtained by this procedure.

Table VII.—Ether-Soluble Extractive by New Method

| Sample Number | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| % ether-soluble extractive | 4.58 | 4.69 | 4.77 | 4.70 | 4.67 | 4.53 |

## SUMMARY AND CONCLUSIONS

1. The U. S. P. XI method for the assay of ginger is unsatisfactory. The reason for the failure of operators to obtain concordant results is mainly that the point at which all of the odor of ether is absent is almost impossible to ascertain. Different individuals also vary in their sensitivity toward the odor of ether.

2. Part of the ether-soluble extractive has been shown to be volatile.

3. Longer exposure of the ether-soluble extractive to water-bath temperature will cause a greater loss of weight. As a consequence, the amount of ether-soluble extractive in the sample of ginger may fall below the range of the standard of the U. S. P. XI.

4. Ginger should be stored in air-tight containers in a cool place.

5. The pungent material of ginger is either volatilized or oxidized at temperatures around 100° C.

6. Wirth's method for the determination of the volatile material proved unsatisfactory because the drug could not be dried to a constant weight.

7. Clevenger's method for the determination of the volatile oil content proved satisfactory. However, his methods for the determination of other constants of ginger extracts were too involved and necessitated the use of special equipment.

8. Ginger extracts give a specific color reaction.

9. A more satisfactory method of assay is proposed for the determination of the ether-soluble extractive. The procedure is as follows:

A paper thimble is extracted with ether for two hours in a Soxhlet extraction apparatus. The thimble is then placed in a special glass-stoppered weighing bottle, into which the thimble fits, and, with cover removed, placed in an oven at 100° C. for one hour to volatilize the ether. The cover is then put on the weighing bottle and it is cooled in a desiccator and weighed. The drug, previously dried in a sulfuric acid desiccator for 48 hours, is then placed in the thimble and weighed and is extracted with ether for six hours. The thimble and drug are then placed in the glass-

stoppered weighing bottle and, with cover removed, placed in an oven at 100° C. for one hour to volatilize the ether. The cover is then put on the weighing bottle and it is cooled in a desiccator and weighed. The loss in weight of the drug represents the ether-soluble extractive.

BIBLIOGRAPHY

(1) Stenhouse, J., *Pharm. J.*, 37 (1877), 70.
(2) Thresh, J. C., *Ibid.*, 41 (1881), 243.
(3) Kilmer, F. B., *Am. J. Pharm.*, 70 (1898), 65.
(4) Von Soden, H., and Rojahn, W., PROC. AM. PHARM. ASSOC., 49 (1901), 821.
(5) Garnett, H., and Grier, J., *Yr. Bk. Pharm., Brit.*, 40 (1907), 443.
(6) Kraemer, H., and Sindall, H., *Am. J. Pharm.*, 80 (1908), 303.
(7) Gildemeister, E., "The Volatile Oils," Second Edition (1913), page 109.
(8) Harrison, C. W., and Sullivan, A. L., *J. Assoc. Official Agr. Chem.*, 1 (1906), 506.
(9) Brooks, B. T., *J. Am. Chem. Soc.*, 38 (1916), 430–432.
(10) Grier, J., *Pharm. J.*, 99 (1917), 172, 205, 216.
(11) Nelson, E. K., *J. Am. Chem. Soc.*, 39 (1917), 1466.
(12) Nomura, H., *Chem. Abs.*, 11 (1917), 2662.
(13) Nomura, H., *Drug. Circ.*, 63 (1919), 552.
(14) Ewe, G. E., JOUR. A. PH. A., 9 (1920), 309.
(15) Clevenger, J. F., *Ibid.*, 17 (1928), 345.
(16) Redgrove, H. S., *Pharm. J.*, 125 (1930), 54.
(17) Munch, J. C., and Byers, G. E., and Pratt, H. J., JOUR. A. PH. A., 23 (1934), 24.
(18) Meek, H., and Salvin, F. G., *Quart. J. Pharm. and Pharmacol.*, 10 (1937), No. 3, 471.
(19) Tzucker, R. T., Thesis: "A Study of the Assay of Ginger (1938)," Purdue School of Pharmacy Library.

NOTICE

Authors who desire to make corrections in papers read at the Richmond meeting, please do so promptly so that the manuscript may be sent to the Board of Review without delay.

# Non-Interfering Adsorbents in Alkaloidal Analysis*

*By Estelle Koozin Johnson and L. Wait Rising*†

The purpose of this investigation was to find adsorbents that would be applicable in toxicological analysis involving alkaloids. An agent which coagulates protein material and removes pigments and dyes, but which does not adsorb any dissolved alkaloid, would not only facilitate filtration and clarification but would also eliminate digestion with heat and, therefore, would be especially applicable in analyses involving easily decomposable alkaloids.

Since the discovery by Lloyd (1), in 1910, of the affinity of fuller's earth for alkaloids, much work has been done toward finding an adsorbent which would adsorb alkaloids quantitatively. However, no systematic investigations have been made to determine which adsorbents have no affinity for all or some alkaloids and under what conditions the adsorption is at a minimum.

In this investigation, both the alkaloids and the adsorbents were selected so as to represent different chemical types, limiting the choice to the more commonly used alkaloids and adsorbents. The alkaloids were codeine, quinine, pilocarpine, atropine and cocaine. The adsorbents were talc, kaolin, prime silica gel, alumina cream and Activated Charcoal, U. S. P. XI. Charcoal was included to determine whether it has any selective action whatsoever, and to determine to what extent certain physical conditions affect its adsorptive capacity.

## EXPERIMENTAL

*General Procedure.*—Aqueous solutions of the alkaloidal salts (0.5% and 1.0%) were prepared and the $p_H$ adjusted to definite values. Five-gram and 20-gram portions of the adsorbents were placed in different bottles and 100-cc. portions of the alkaloidal solution were pipetted into each bottle.

* An abstract of a thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy, University of Washington, Seattle, Washington.

† Professor of Pharmacy, University of Washington, Seattle, Washington.

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Table I.—Adsorption of Codeine Sulfate

Assay of dry salt = 101.8% $(C_{18}H_{21}O_3N)_2.H_2SO_4$

| Adsorbent | Contact = 10 Min. 5 Gm. | 20 Gm. | Contact = 3 Hrs. 5 Gm. | 20 Gm. |
|---|---|---|---|---|
| $p_H$ 4.9; 1.0% soln.; max. var. = 1.2% | | | | |
| Talc | 97.7 | 87.0 | 99.2 | 87.7 |
| Kaolin | 96.7 | 84.2 | 98.2 | 87.4 |
| Silica gel | 88.6 | 61.0 | 86.8 | 55.0 |
| Alumina cream | 95.7 | 84.3 | 95.2 | 85.7 |
| Charcoal | 0.3 | 1.1 | 3.1 | 1.6 |
| $p_H$ 3.2; 1.0% soln.; max. var. = 0.6% | | | | |
| Talc | 97.7 | 90.3 | 97.0 | ... |
| Kaolin | 97.3 | 87.5 | 96.5 | ... |
| Silica gel | 88.1 | 56.8 | 84.0 | ... |
| Alumina cream | 95.9 | 88.3 | 95.3 | ... |
| Charcoal | 0.3 | 3.0 | 0.6 | ... |
| $p_H$ 1.6; 1.0% soln.; max. var. = 0.8% | | | | |
| Talc | 99.4 | ... | ... | ... |
| Kaolin | 97.1 | ... | ... | ... |
| Silica gel | 86.8 | ... | ... | ... |
| Alumina cream | 97.3 | ... | ... | ... |
| Charcoal | 1.4 | ... | ... | ... |
| $p_H$ 7.7; 1.0% soln.; max. var. = 0.2% | | | | |
| Talc | 97.4 | ... | ... | ... |
| Kaolin | 97.0 | ... | ... | ... |
| Silica gel | 80.8 | ... | ... | ... |
| Alumina cream | 95.5 | ... | ... | ... |
| Charcoal | 1.5 | ... | ... | ... |
| $p_H$ 4.8; 0.5% soln.; max. var. = 1.1% | | | | |
| Talc | 95.8 | 78.4 | 98.8 | ... |
| Kaolin | 97.9 | 76.2 | 95.7 | ... |
| Silica gel | 88.2 | 57.2 | 85.9 | ... |
| Alumina cream | 98.2 | 81.8 | 97.9 | ... |
| Charcoal | 3.2 | 6.2 | 3.1 | ... |

Table II.—Adsorption of Quinine Dihydrochloride

$C_{20}H_{24}O_2N_2.2HCl$; max. var. = 1.3%

| Adsorbent | Contact = 10 Min. 5 Gm. | 20 Gm. | Contact = 3 Hrs. 5 Gm. | 20 Gm. |
|---|---|---|---|---|
| $p_H$ 6.2; 1.0% soln.; control assay = 102.9% | | | | |
| Talc | 92.5 | ... | ... | ... |
| Kaolin | 91.6 | ... | ... | ... |
| Silica gel | 86.8 | ... | ... | ... |
| Alumina cream | 94.1 | ... | ... | ... |
| Charcoal | 0.0 | ... | ... | ... |
| $p_H$ 3.0; 1.0% soln.; control assay = 95.5% $C_{20}H_{24}O_2N_2.2HCl$; max. var. = 0.6% | | | | |
| Talc | 94.4 | 84.1 | 92.7 | 82.6 |
| Kaolin | 94.9 | 87.1 | 94.2 | 85.7 |
| Silica gel | 94.6 | 78.5 | 91.8 | 71.4 |
| Alumina cream | 94.6 | 86.1 | 94.7 | 86.9 |
| Charcoal | 1.2 | 0.5 | 0.5 | 0.0 |
| $p_H$ 1.0; 1.0% soln.; control assay = 100.1% $C_{20}H_{24}O_2N_2.2HCl$; max. var. = 0.9% | | | | |
| Talc | 97.4 | ... | ... | ... |
| Kaolin | 98.2 | ... | ... | ... |
| Silica gel | 91.6 | ... | ... | ... |
| Alumina cream | 96.3 | ... | ... | ... |
| Charcoal | 0.6 | ... | ... | ... |
| $p_H$ 3.0; 0.5% soln.; control assay = 100.5% $C_{20}H_{24}O_2N_2.2HCl$; max. var. = 0.9% | | | | |
| Talc | 92.4 | ... | ... | ... |
| Kaolin | 92.7 | ... | ... | ... |
| Silica gel | 92.5 | ... | ... | ... |
| Alumina cream | 95.7 | ... | ... | ... |
| Charcoal | 1.3 | ... | ... | ... |

The bottles were vigorously shaken on a mechanical shaker for ten minutes and three hours. The mixtures were filtered, 25-cc. aliquot portions of the filtrate were pipetted into separatory funnels and the residual alkaloid was extracted by the usual method, using chloroform as the immiscible solvent. The chloroform extracts were evaporated, the residues dried at 100–105° C. and the percentages of recovery were calculated.

*Results.*—The figures in Tables I to V represent the percentages of recovery and are expressed as alkaloidal salt. Codeine sulfate, which contains 5 molecules of water of crystallization, was dried and assayed before being used. Solutions prepared from it were exactly 0.5% or 1.0% in strength, calculated as $(C_{18}H_{21}O_3N)_2.H_2SO_4$. The other alkaloidal salts were used without drying. Solutions were prepared approximately 0.5% and 1.0% in strength and control analyses were conducted. The calculations for both the controls and the adsorption determinations, were based on the assumption that the solutions contained exactly 0.5% or 1.0% of the alkaloidal salt.

Table III.—Adsorption of Pilocarpine Hydrochloride

| Adsorbent | Contact = 10 Min. 5 Gm. | 20 Gm. | Contact = 3 Hrs. 5 Gm. | 20 Gm. |
|---|---|---|---|---|
| $p_H$ 7.0; 1.0% soln.; control assay = 94.4% $C_{11}H_{16}O_2N_2.HCl$; max. var. = 1.5% | | | | |
| Talc | 92.5 | ... | ... | ... |
| Kaolin | 90.8 | ... | ... | ... |
| Silica gel | 64.0 | ... | ... | ... |
| Alumina cream | 91.8 | ... | ... | ... |
| Charcoal | 0.3 | ... | ... | ... |
| $p_H$ 4.5; 1.0% soln.; control assay = 96.5% $C_{11}H_{16}O_2N_2.HCl$; max. var. = 1.3% | | | | |
| Talc | 90.6 | 87.9 | 92.4 | 89.7 |
| Kaolin | 92.2 | 88.2 | 93.0 | 90.5 |
| Silica gel | 87.7 | 67.9 | 86.0 | 69.5 |
| Alumina cream | 91.0 | 82.6 | 90.1 | 84.2 |
| Charcoal | 3.4 | 0.5 | 4.7 | 1.2 |
| $p_H$ 1.0; 1.0% soln.; control assay = 96.7% $C_{11}H_{16}O_2N_2.HCl$; max. var. = 1.2% | | | | |
| Talc | 93.0 | ... | ... | ... |
| Kaolin | 93.7 | ... | ... | ... |
| Silica gel | 80.9 | ... | ... | ... |
| Alumina cream | 89.9 | ... | ... | ... |
| Charcoal | 0.0 | ... | ... | ... |
| $p_H$ 4.5; 0.5% soln.; control assay = 89.6% $C_{11}H_{16}O_2N_2.HCl$; max. var. = 0.8% | | | | |
| Talc | 88.6 | ... | ... | ... |
| Kaolin | 89.0 | ... | ... | ... |
| Silica gel | 84.2 | ... | ... | ... |
| Alumina cream | 89.2 | ... | ... | ... |
| Charcoal | 0.5 | ... | ... | ... |

Table IV.—Adsorption of Atropine Sulfate

| Adsorbent | Contact = 10 Min. 5 Gm. | 20 Gm. | Contact = 3 Hrs. 5 Gm. | 20 Gm. |
|---|---|---|---|---|
| $p_H$ 5.9; 1.0% soln.; control assay = 99.2% $(C_{17}H_{23}O_3N)_2.H_2SO_4.H_2O$; max. var. = 0.9% | | | | |
| Talc | 96.8 | 88.5 | 94.6 | 85.3 |
| Kaolin | 96.5 | 84.1 | 94.9 | 84.0 |
| Silica gel | 84.1 | 54.0 | 82.3 | 49.0 |
| Alumina cream | 95.2 | 82.1 | 93.6 | 83.2 |
| Charcoal | 2.7 | 0.7 | 1.3 | 0.2 |

Table IV (*Continued*)

| Adsorbent | Contact = 10 Min. 5 Gm. | 20 Gm. | Contact = 3 Hrs. 5 Gm. | 20 Gm. |
|---|---|---|---|---|
| $p_H$ 3.0; 1.0% soln.; control assay = 96.8% $(C_{17}H_{23}O_3N)_2.H_2SO_4.H_2O$; max. var. = 0.7% | | | | |
| Talc | 93.6 | ... | ... | ... |
| Kaolin | 93.2 | ... | ... | ... |
| Silica gel | 82.2 | ... | ... | ... |
| Alumina cream | 92.4 | ... | ... | ... |
| Charcoal | 0.6 | ... | ... | ... |
| $p_H$ 1.0; 1.0% soln.; control assay = 96.8% $(C_{17}H_{23}O_3N)_2.H_2SO_4.H_2O$; max. var. = 0.3% | | | | |
| Talc | 93.5 | ... | ... | ... |
| Kaolin | 93.3 | ... | ... | ... |
| Silica gel | 80.1 | ... | ... | ... |
| Alumina cream | 93.9 | ... | ... | ... |
| Charcoal | 0.4 | ... | ... | ... |
| $p_H$ 5.9; 0.5% soln.; control assay = 97.2% $(C_{17}H_{23}O_3N)_2.H_2SO_4.H_2O$; max. var. = 0.4% | | | | |
| Talc | 92.6 | ... | ... | ... |
| Kaolin | 91.5 | ... | ... | ... |
| Silica | 81.8 | ... | ... | ... |
| Alumina cream | 92.7 | ... | ... | ... |
| Charcoal | 0.6 | ... | ... | ... |

Table V.—Adsorption of Cocaine Hydrochloride

| Adsorbent | Contact = 10 Min. 5 Gm. | 20 Gm. | Contact = 3 Hrs. 5 Gm. | 20 Gm. |
|---|---|---|---|---|
| $p_H$ 6.7; 1.0% soln.; control assay = 99.1% $C_{17}H_{21}O_4N.HCl$; max. var. = 0.7% | | | | |
| Talc | 93.2 | ... | ... | ... |
| Kaolin | 95.3 | ... | ... | ... |
| Silica gel | 91.3 | ... | ... | ... |
| Alumina cream | 96.7 | ... | ... | ... |
| Charcoal | 0.2 | ... | ... | ... |
| $p_H$ 4.6; 1.0% soln.; control assay = 100.1% $C_{17}H_{21}O_4N.HCl$; max. var. = 1.1% | | | | |
| Talc | 93.9 | ... | 75.1 | ... |
| Kaolin | 96.7 | ... | 86.1 | ... |
| Silica gel | 92.1 | ... | 59.6 | ... |
| Alumina cream | 97.9 | ... | 86.2 | ... |
| Charcoal | 0.8 | ... | 0.0 | ... |
| $p_H$ 1.0; 1.0% solution; control assay = 101.4% $C_{17}H_{21}O_4N.HCl$; max. var. = 1.7% | | | | |
| Talc | 97.4 | ... | ... | ... |
| Kaolin | 98.5 | ... | ... | ... |
| Silica gel | 87.9 | ... | ... | ... |
| Alumina cream | 94.9 | ... | ... | ... |
| Charcoal | 0.2 | ... | ... | ... |

SUMMARY

1. The adsorption of codeine, quinine, atropine, pilocarpine and cocaine by talc, kaolin, silica gel, alumina cream and charcoal was investigated.

2. Adsorption of the alkaloids by all five adsorbents was practically unaffected by changes in $p_H$ of the original solutions. Adsorption of pilocarpine by silica gel was an exception. In this case a considerably greater per cent of the alkaloid was adsorbed at $p_H$ 7.0 than at either $p_H$ 1.0 or 4.5, the lowest per cent being adsorbed at $p_H$ 4.5.

3. Increasing the period of contact caused a slight increase in adsorption by silica gel. The adsorption by the other agents remained practically constant.

4. Increasing the weight of adsorbent caused a definite increase in the amount of adsorption of the five alkaloids by all adsorbents. The increase was especially marked in the case of silica gel.

5. Concentration of the solutions had only a slight effect.

6. The affinity of any one adsorbent for the five alkaloids varied only within narrow limits.

7. The adsorption by charcoal was complete or practically complete under all conditions. Adsorption by silica gel was much less than by charcoal, but, in general, greater than by the other adsorbents.

8. Adsorption by talc, kaolin and alumina cream was negligible when 5 Gm. of the adsorbents were used. With 20 Gm. adsorption increased sufficiently to interfere with quantitative recovery.

REFERENCE

(1) Lloyd, J. U., JOUR. A. PH. A., 5 (1916), 381.

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# The Preparation of Magnesium Silicates by the Interaction of Magnesium Salts and Alkali Metal Silicates*

*By R. Roseman, H. Eisenberg and M. B. Levin*†

The recent publications of Mutch (1–4), in England, and Levin and co-workers (5, 6), in this country, describing the usefulness of silicates of magnesium in clinical medicine (peptic ulcer therapy), have aroused considerable interest in these materials, as a number of other publications (7) will attest.

The primary purpose of the present paper is to review the literature on the wet reaction between magnesium sulfate (or chloride) and sodium (or potassium) silicate. It is hoped that those interested may thereby gain a better insight into the nature of this

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† The Burton-Levin Foundation, Inc., Baltimore, Maryland.

complex chemical process, and so acquire a more intelligent approach to the problem of preparing various silicates of magnesium for their researches.

The problem is certainly not to be dismissed with the statement, "Alkaline silicates precipitate gelatinous magnesium silicate from magnesium salts; the precipitate is apparently $MgO.2SiO_2.2H_2O$" (8). And, in the following paragraphs are given, chiefly by means of quotations from the original publications, all available details concerning materials and procedures employed and products obtained, together with observations of interest. Comments of our own are also included.

DÖBEREINER (9)

It is just one hundred years since Döbereiner published his paper, "Analyse und Synthese des Meerschaums." The article is here reproduced in its entirety (Fig. 1).

Döbereiner (9) writes:

"Von einem anderen meiner Zuhörer, Hrn. Gundelach aus Ilmenau, liess ich versuchen, Meerschaum [a hydrous magnesium silicate in which the molecular ratio, $MgO:SiO_2 = 2:3$] künstlich darzustellen durch Fällung einer sehr verdünnten Auflösung von schwefelsaurer Magnesia mit einer ebenfalls sehr diluirten Solution von kieselsaurem Kali ($KO + SiO_3$) [$2K_2O,3SiO_2$?]. Der Versuch gelang vollkommen, wir erhielten einen Niederschlag von kleisterartiger Beschaffenheit, welcher nach oft wiederholtem Auswaschen in einer grossen hohen Kapsel von Saugpapier, an der Luft langsam getrock-

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Auge nicht so angenehm war, als wenn ich einen Stempel gehabt hätte, um sie mit erhabenen Figuren auszudrücken. Dieses Verfahren bietet den Vortheil dar, dass, wenn man das anhängende Eisen von Neuem darüber bringt, alles ausgelöscht wird, ohne dass die Substanz beim Schmelzen verbrennt, noch irgend eine Veränderung erleidet, und dass man, da sie von Neuem dazu gebraucht werden kann, von einer bestimmten Menge dieser Zusammensetzung nach und nach bis auf 40 verschiedene Abdrücke mit verschiedenen Stempeln erhalten kann.

Wird Jod allein in dieselben Umstände versetzt wie das Jodchlorid, so erzeugt es selbst binnen 14 Tagen keine merkliche Wirkung auf Kork.

Man könnte diese neue Zusammensetzung Suberen nennen, wenn dieser Name nicht dem ölartigen Körper gegeben worden wäre, den Boussingault bei der Destillation von korksaurem Kalk erhielt. Uebrigens wird die Elementaranalyse, welche die Chemiker damit anstellen werden, da ich sie selbst nicht vornehmen konnte, ohne Zweifel einen passenden Namen darbieten, der zugleich die Zusammensetzung und den Ursprung dieser neuen organischen Substanz ausdrückt.

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## XXV.

## *Analyse und Synthese des Meerschaums.*

Von

J. W. DÖBEREINER.

Von Hrn. Eisenach aus Weimar liess ich in diesem Winter den im Handel vorkommenden orientalischen Meerschaum analysiren. Derselbe fand darin 1 At. Magnesia, 1 At. Kieselsäure ($SiO_3$) und 2 At. Wasser, von letzterem also 1 At. mehr als Lychnell erhalten. Ich fand dabei, dass der bei der glühenden Hitze entwässerte Meerschaum sich stark erwärmt, wenn man ihn einige Augenblicke in kaltes Wasser taucht, und dass er auch an feuchter Luft sehr bald wieder die ganze Menge des verlornen Hydratwassers anzieht, dass er aber durch starkes Glühen die Eigenschaft verliert, sich beim Befeuchten mit Wasser zu erwärmen.

Von einem anderen meiner Zuhörer, Hrn. Gundelach aus Ilmenau, liess ich versuchen, Meerschaum künstlich darzu-

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stellen durch Fällung einer sehr verdünnten Auflösung von schwefelsaurer Magnesia mit einer ebenfalls sehr diluirten Solution von kieselsaurem Kali ($KO + SiO_3$). Der Versuch gelang vollkommen, wir erhielten einen Niederschlag von kleisterartiger Beschaffenheit, welcher nach oft wiederholtem Auswaschen in einer grossen hohen Kapsel von Saugpapier, an der Luft langsam getrocknet, eine leichte, fest zusammenhängende schwammige Masse darstellte. Hr. Gundelach ist im Begriff, diesen Versuch sehr im Grossen zu wiederholen, um zu erfahren, um berechnen zu können, ob die Fabrication des Meerschaums als vortheilhaftes Unternehmen werden könne.

Reibt man den natürlichen oder künstlichen Meerschaum mit wenig Wasser, so verwandelt er sich in eine steife kleisterartige Masse. Vermengt man diese Masse mit Platinsalmiak, so erhält man einen gelben Teig, woraus Kugeln, Schälchen, Tiegelchen u. s. w. geformt werden können. Trocknet man diese geformten Massen langsam an der Luft, erhitzt dann dieselben nach und nach bis zum Glühen und erhitzt lange als sich noch riechende Dämpfe entwickeln, so werden sie schwarzgrau, ohne ihre Form zu verlieren. Diese geformten Massen werden fast augenblicklich glühend, wenn man sie unter dem Zutritte der Luft einem Strome von Wasserstoffgas aussetzt, bestimmen den Alkohol, sich zu oxydiren, wobei sie, wenn sie noch kein hygroskopisches Wasser enthalten, glühend werden, und wirken auf gewöhnliches Knallgas, d. h. auf eine Mischung von 2 Vol. atmosphärischer Luft und 1 Vol. Wasserstoffgas, bei weitem energischer als meine mit Töpferthon bereiteten eudiometrischen Platinpillen. Besonders überraschend ist es zu sehen, wie ein auf den Mittelpunct eines meerschaumenen Platinschälchens geleiteter dünner Strom von Hydrogengas diesen Punct plötzlich hellglühend macht, während die übrige Oberfläche dunkel oder finster bleibt.

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Fig. 1.—The Hundred-Year Old Publication of Döbereiner, *J. prakt. Chem.*, 17 (1839) 157.

net, eine leichte, fest zusammenhängende schneidbare Masse darstellte."

LEFORT (10)

Lefort, in his "Mémoire sur les silicates," writes: "Le silicate de potasse et le sulfate de magnésie donnent lieu à un abondant précipité blanc qui a la plus grande resemblance avec l'hydrocarbonate de magnésie. Ce sel a pour composition: $3(SiO^2)$,-MgO,2HO." (The precipitate was dried at 150–200°.)

Lefort believed that "une condition indispensable pour obtenir des silicates terreux ayant une composition définie, consiste à opérer avec des solutions étendues," and that "il est de toute nécessité que les sels terreux qui doivent être employés à la préparation des silicates terreux soient parfaitement neutres." Furthermore, the precipitated silicates "doivent être lavés avec soin et avec de l'eau distillée bouillie froide afin de les obtenir purs."

Lefort gives "$3(SiO^2)$,KO,9HO" as the potassium silicate employed in his researches.

At the time of the publication of the "Mémoire," the following atomic weights were apparently in use: H = 1, O = 8, Mg = 12, Si = 21 and K = 39.1 (see Kopp-Will's "Jahresbericht," 1861, p. xxi). It would seem, then, that in Lefort's magnesium (or potassium) silicate, the molecular ratio, MgO (or $K_2O$):$SiO_2$, was 1:4.5.

VON AMMON (11)

Although the Library of Congress, Washington, D. C., could find no record of a copy of Von Ammon's work in the United States, summaries appear in Kopp-Will's "Jahresbericht" (11) and Gmelin-Kraut's "Handbuch" (11), stating, in part:

"Der Weg, welchen Ammon zur Darstellung der Silicate der Erden einschlug, bestand darin, dass Er die meist verdünnte Lösung eines Erdsalzes mit der ebenfalls verdünnten des kiesels. Natronsnie derschlug."

"$MgSiO_3$.—Durch Doppelzersetzung von $MgSO_4$ mit überschüssigem kristallisiertem Natriumsilikat. —Weisse, sehr voluminöse Gallerte, nach dem Trocknen bald pulvrig, bald dicht und opalartig." (The sodium silicate employed was apparently $Na_2SiO_3.8H_2O$.)

Two sets of analyses are given, as follows:

| | (*a*) | (*b*) |
|---|---|---|
| Per cent MgO | 29.04 | 29.84 |
| Per cent $SiO_2$ | 46.95 | 47.31 |
| Per cent $H_2O$ | 22.54 | 22.58 |

The molecular ratios, MgO:$SiO_2$, are 1:1.09 and 1:1.06 (average = 1:1.08).

HELDT (12)

According to the Heldt publication, "Wird eine Auflösung von Bittersalz mit Wasserglas [apparently $K_2O$,$3SiO_2$] versetzt, so fällt sogleich ein weisser, gelatinöser Niederschlag, der nach 8 tägigem Stehen in der Flüssigkeit nicht krystallinisch wurde." After drying at 100°, the material was analyzed, yielding 17.0 per cent MgO, 71.4 per cent $SiO_2$, and 12.7 per cent $H_2O$. Here, the molecular ratio, MgO:$SiO_2$ = 1:2.82, so that Heldt's product was not the "single compound, $MgO.2SiO_2.2H_2O$," given by Kardos and Joffe (19).

"Es wurden nun noch basische Silicate der Magnesia gefällt, indem vorher die Wasserglaslösung mit verschiedenen Mengen Kalilösung versetzt wurde, * * *."

HAUSHOFER (13)

Included in a group of synthetic silicates obtained by Haushofer was a material containing 14.4 per cent MgO, 68.2 per cent $SiO_2$ and 17.2 per cent $H_2O$, which was prepared by the action of "Kaliwasserglas" on magnesium sulfate. "Die Niederschläge wurden nach längerem Stehenlassen gut ausgewaschen, mit destillirtem Wasser mehrmals ausgekocht und decantirt, endlich filtrirt; es war meine Absicht, auf diese Weise mechanisch anhaftendes Kali möglichst vollständig zu entfernen."

Haushofer closes his communication with the statement: "Ich vermuthe, dass bei allen diesen Fällungen freie Kieselerde oder ein Hydrat von Kieselerde ausfällt; es ist jedoch sehr schwer, darüber Gewissheit zu erhalten."

It should be pointed out that Haushofer's product, of molecular ratio, MgO:$SiO_2$ = 1:3.18, was not the "$Mg_2Si_7O_{16}.2H_2O$," given by Mutch (1).

MAY (14)

May's technique for preparing a number of silicates follows:

"Die Auflösungen der Metallsalze wurden so lange in die Lösung des kieselsauren Natrons getropft, als noch ein Niederschlag entstand. Die Niederschläge wurden sorgsam ausgewaschen, bei 100° getrocknet * * *."

By proceeding in the above manner, and employing (*a*) a magnesium sulfate solution of specific gravity 1.052, and (*b*) a sodium silicate solution of molecular ratio, $Na_2O$:$SiO_2$ = 1:1.00, and specific gravity 1.079, May obtained a product containing 28.6 per cent MgO, 49.4 per cent $SiO_2$ and 22.4 per cent $H_2O$. ("In der von der Magnesia abfiltrirten Flüssigkeit fand ich kein Alkali.") In this substance, the molecular ratio, MgO:$SiO_2$ = 1:1.16.

GLASS (15)

Glass states that "if to any of these materials [silicates of soda] an excess of magnesium sulfate solution be added it will be found that all the silica is thrown out of solution together with, roughly speaking, as much magnesium as is the equivalent of the sodium present."

Glass writes further that "the examination of a number of precipitates obtained by the action of magnesium sulfate upon sodium silicate solutions indicated that the primary effect of the addition of a metallic salt was the precipitation of silica; this silica then combined with, or absorbed, or occluded,

a certain amount of magnesia, and the extent of this depends upon the opportunity for contact and reaction."

Table I.—Analyses of Glass's Preparations

| Experiment | Per Cent MgO[a] | Per Cent $SiO_2$[a] | Molecular Ratio, MgO:$SiO_2$ |
|---|---|---|---|
| 1 | 20.54 | 78.50 | 1:2.57 |
| 2 | 20.42 | 78.96 | 1:2.60 |
| 3 | 30.78 | 68.64 | 1:1.50 |
| 4 | 30.57 | 69.61 | 1:1.53 |
| 5 | 24.95 | 74.85 | 1:2.01 |
| 6 | 24.20 | 75.80 | 1:2.10 |
| 7 | 29.15 | 69.31 | 1:1.60 |

[a] Ignited basis.

The solutions of sodium silicate used in Experiments 1 and 2 were made from a "crystalline compound" of molecular ratio, $Na_2O:SiO_2 = 1:2.62$ (23.1 per cent $Na_2O$, 58.6 per cent $SiO_2$).

The precipitate of Experiment 1 was prepared "from equal weights of the silicate and magnesium sulfate, the magnesium sulfate solution being slowly run into that of the silicate." The ratio, moles $Na_2O,2.62SiO_2$/moles $MgSO_4.7H_2O$, was then 0.92.

In Experiment 2, "nine parts of magnesium sulfate were used to ten parts of silicate, and, after precipitation, the whole was evaporated to dryness, ground up and washed." Here, the ratio, moles $Na_2O,2.62SiO_2$/moles $MgSO_4.7H_2O$, was 1.02.

Experiment 3 was a repetition of Experiment 2, but enough caustic soda was added to the $Na_2O,2.62SiO_2$ to give $Na_2O,1.50SiO_2$, and equimolecular amounts of the resulting $Na_2O,1.50SiO_2$ and $MgSO_4.7H_2O$ were employed. The object of this experiment, as well as of Experiments 4 and 7, was to prepare a synthetic silicate of molecular ratio, MgO:$SiO_2$ = 1:1.50 (2:3).

Experiment 4 was the same as Experiment 3 "except that the precipitate was washed straight away instead of first evaporating to dryness."

A "similar series of experiments" was made using a liquid sodium silicate of molecular ratio, $Na_2O:SiO_2$ = 1:2.06 (36.6 per cent $SiO_2$). Experiments 5 and 6 were made without the addition of caustic soda; in Experiment 7, equimolecular amounts of $Na_2O,1.50SiO_2$—prepared by the addition of the requisite amount of NaOH to the $Na_2O,2.06SiO_2$—and $MgSO_4.7H_2O$ were employed.

Mutch (4) writes that "Glass's studies are deprived of their scientific interest by his use of inaccurate methods of analysis." Yet, in the same paper (4), Mutch's own analyses of various brands of "magnesium trisilicate" are seen to result in summations ranging from 97.18 per cent to 107.03 per cent.

ROSEMAN AND CO-WORKERS (16)

"The primary purpose of the present investigation was to ascertain the extent of the variation in chemical composition of the products obtained when varying proportions of sodium silicate and magnesium sulfate are allowed to interact."

Table II.—Résumé of Experiments

| | Experiment[a] | Moles $Na_2O,3.27SiO_2$ / Moles $MgSO_4.7H_2O$ | Per Cent MgO, $H_2O$-Free Basis | Per Cent $SiO_2$, $H_2O$-Free Basis[b] | Molecular Ratio, MgO:$SiO_2$ |
|---|---|---|---|---|---|
| Preliminary Series | 1 | (0.55) | 16.59 | 82.55 | 1:3.24 |
| | 2 | (1.11) | 16.12 | 82.70 | 1:3.44 |
| | 3 | (1.66) | 13.38 | 84.44 | 1:4.24 |
| Final Series | 4 | 0.51 | 17.80 | 81.62 | 1:3.08 |
| | 5 | 0.76 | 17.39 | 82.06 | 1:3.17 |
| | 6 | 1.02 | 16.82 | 82.42 | 1:3.29 |
| | 7 | 1.53 | 15.00 | 84.30 | 1:3.77 |
| | 8 | 2.03 | 13.41 | 86.03 | 1:4.31 |

[a] All of the experiments were conducted at room temperature (approximately 24.5° C.).

[b] Complete analyses ($SiO_2$, MgO, $H_2O$, $R_2O_3$, CaO, $Na_2O$ and $SO_3$) are given in the original publication.

In the final series of experiments (4, 5, 6, 7 and 8, Table II), *varying* amounts of a 1:3.13 (by volume) water solution of sodium silicate of molecular ratio, $Na_2O:SiO_2$ = 1:3.27 (8.86 per cent $Na_2O$, 28.06 per cent $SiO_2$, specific gravity = 1.384 at 25° C.),[1] were added to *fixed* quantities of a 1:30 (by weight) water solution of Baker's C.P. $MgSO_4.7H_2O$, the proportions employed being given in column 2, Table II. The sodium silicate solutions were added slowly (5–20 minutes) from a graduate, with stirring (by motor), stirring being then continued for an additional period (5 minutes) to insure thorough mixing. The precipitates were purified by long washing with distilled water, then dried, powdered and analyzed.

But for the use of tap water in the preliminary series of experiments (1, 2 and 3, Table II), the technique employed in this series was essentially that of the final series.

Table III.—Nomenclature of the Silicic Acids and Silicates

| Type | | 1 Mono- | 2 Di- | 3 Tri- | 4 Tetra- | 5 Penta- |
|---|---|---|---|---|---|---|
| Ortho- | $H_{2n+2}Si_nO_{3n+1}$ | $H_4SiO_4$ | $H_6Si_2O_7$ | $H_8Si_3O_{10}$ | $H_{10}Si_4O_{13}$ | $H_{12}Si_5O_{16}$ |
| Meta-(proto-) | $H_{2n}Si_nO_{3n}$ | $H_2SiO_3$ | $(H_4Si_2O_6)$ | $(H_6Si_3O_9)$ | $(H_8Si_4O_{12})$ | $(H_{10}Si_5O_{15})$ |
| Meso-(deutero-) | $H_{2n-2}Si_nO_{3n-1}$ | ... | $H_2Si_2O_5$ | $H_4Si_3O_8$ | $H_4Si_4O_{11}$ | $H_6Si_5O_{14}$ |
| Para-(tritero-) | $H_{2n-4}Si_nO_{3n-2}$ | ... | ... | $H_2Si_3O_7$ | $H_4Si_4O_{10}$ | $H_6Si_5O_{13}$ |
| Tetrero- | $H_{2n-6}Si_nO_{3n-3}$ | ... | ... | ... | $H_2Si_4O_9$ | $H_4Si_5O_{12}$ |
| Pentero- | $H_{2n-8}Si_nO_{3n-4}$ | ... | ... | ... | ... | $H_2Si_5O_{11}$ |

In column 2, Table II, "the ratios given in parentheses were obtained by assuming that the values actually found for the specific gravity (1.384, 25° C.)

[1] The sodium silicate was "E" Brand of the Philadelphia Quartz Company. "The manufacturers of this material state that it is a 40° Baumé liquid holding, according to a typical analysis, 8.70 per cent $Na_2O$, 28.06 per cent $SiO_2$, 0.079 per cent $Al_2O_3$, 0.015 per cent $Fe_2O_3$, 0.036 per cent $TiO_2$, 0.020 per cent CaO, 0.042 per cent MgO, 0.035 per cent Cl, 0.025 per cent $SO_3$, 0.038 per cent $CO_2$ (molecular ratio, $Na_2O:SiO_2$ = 1:3.33)."

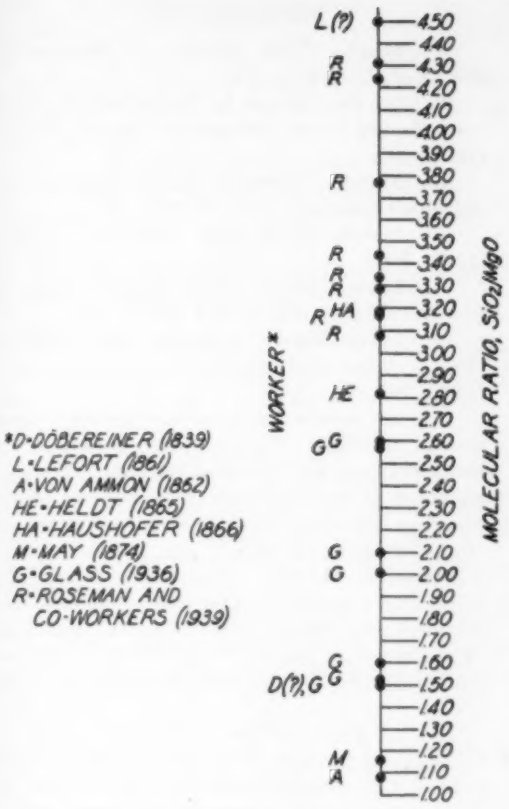


Fig. 2.—Magnesium Silicates Prepared by Various Workers.

and the $Na_2O$ and $SiO_2$ contents (8.86 per cent and 28.06 per cent, respectively) of the sample of 'E' Brand sodium silicate used in the final series of experiments were applicable to the previously purchased and unanalyzed sample of 'E' Brand employed in the preliminary series of experiments."

From the data obtained in the final series of experiments, it is seen that "with increasing ratios of the amounts of reacting substances, moles $Na_2O$,-$3.27SiO_2$/moles $MgSO_4.7H_2O$, the products show progressively increasing silica contents and correspondingly decreasing magnesia contents (water-free basis), with the attendant increasing ratios, moles $SiO_2$/moles MgO."

Furthermore, interpolation of these data will show that "a synthetic magnesium silicate, in which the molecular ratio, $MgO:SiO_2$, is the same as the molecular ratio, $Na_2O:SiO_2$, in the sodium silicate used in its preparation (1:3.27), results from the interaction of equal numbers of moles of the sodium silicate and magnesium sulfate."

The noteworthy amounts of calcium found in the products of Experiments 1, 2 and 3 (0.29, 0.26 and 0.96 per cent CaO, respectively), more especially Experiment 3, "may be derived from the tap water used in these experiments by base-exchange, by adsorption, and as the result of contamination by precipitated calcium silicate coming from the interaction of dissolved calcium compounds and sodium silicate."

The average $Na_2O$ and $SO_3$ contents (for the products of Experiments 1–8) being 0.03 per cent and 0.01 per cent, respectively, it was concluded that "thorough washing of the precipitated magnesium silicates enables one to obtain preparations that are essentially free of sodium (and sulfate), even where a considerable excess of sodium silicate has been employed as precipitant."

### OTHER WORK

Britton (17), in his "Electrometric Study of the Precipitation of Silicates," found, among other things, that "the precipitates obtained by the interaction of a solution of sodium silicate, $Na_2O$,2.16-$SiO_2$, and metallic-salt solutions, except that of calcium chloride, contained less silica than that required to form the metasilicates." (The sodium silicate solution, which was 0.1020*N* with respect to sodium hydroxide, was added to the magnesium sulfate solution, which was 0.02*M*.)

Joffe and co-workers (18, 19) thought it desirable, "in view of the conflicting character of the available data, * * * to investigate the nature of the compounds of the alkaline earths with silica from the standpoint of stability and ionic affinity with careful control of the $p_H$ of the reacting medium." For a thorough understanding of this excellent work, the reader, preferably familiar with the methods of soil chemistry, is referred to the original publications.

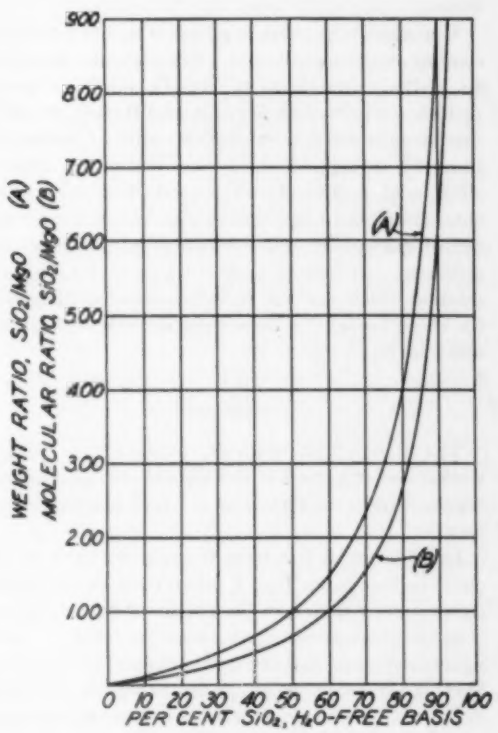


Fig. 3.—Idealized Curves.

Finally, a number of additional contributions (20–45) are included in the bibliography, by reason of their pertinence or other interest.

## NOMENCLATURE

Concerning nomenclature, Mellor (46) writes:

"The silicates can be regarded as salts of silicic acid and its various condensation products. The general formula of all the silicic acids is * * * $H_{2m}Si_nO_{2n+m}$, where $n$ denotes the number of silicic atoms and $m$, half of the hydrogen atoms per mol. The silicic acids can be named by starting from the *orthosilicic acids*, the acids formed by the loss of one mol of water per mol of acid can be called *protosilicic acids* or, as is more usually the case, *metasilicic acids;* the loss of two mols of water gives *deuterosilicic acids* or *mesosilicic acids;* the loss of three mols of water gives the *triterosilicic acids* or *parasilicic acids;* the loss of four mols of water gives the *tetrerosilicic acids* and the loss of five mols of water gives the *penterosilicic acids*."

These acids are illustrated in Table III (Mellor's Table X, with our corrections), "which can be extended to the right and downward if occasion should arise. Each member of a horizontal row differs from the one which precedes by the addition of $H_2SiO_3$; and each member of a vertical column differs from the one which precedes by the removal of $H_2O$. The various metasilicic acids are polymers of one another; and those formulas enclosed by brackets in the table are polymers of some other member of the series."

Unfortunately, there is a lack of agreement in the naming of these materials. For example, according to Mellor's classification, $H_4Si_2O_5$ would be metadisilicic (or protodisilicic) acid, and $H_2Si_2O_5$ would be mesodisilicic (or deuterodisilicic) acid. Clarke (47), however, would call the former compound dimetasilicic acid, and the latter, metadisilicic acid. Further, Hildebrand (48) writes that "there are a number of poly-silicic acids, which require such distinguishing terms as di-meta-silicic acid for $H_2Si_2O_5$; tri-meta-silicic acid for $H_2Si_3O_7$; di-ortho-silicic acid for $H_4Si_2O_5$, etc.," in disagreement with both Mellor and Clarke.

## CONCLUSION

The magnesium silicates, prepared by various workers as described in this paper, are conveniently represented as shown in Fig. 2, which is self-explanatory.

In addition, it has been thought desirable to include in this paper Fig. 3, comprising two idealized curves which show how, in a series of pure synthetic silicates of magnesium, the $SiO_2$ percentages, on a water-free basis, and the corresponding ratios, $SiO_2$/MgO, are related, weight ratios being the ordinates in Curve (A), while molecular ratios are the ordinates in Curve (B). The curves are portions of equilateral hyperbolas.

## BIBLIOGRAPHY

(1) Mutch, "The Silicates of Magnesium," *Brit. Med. J.*, 1 (1936), 143.

(2) Mutch, "Synthetic Magnesium Trisilicate—Its Action in the Alimentary Tract," *Ibid.*, 1 (1936), 205.

(3) Mutch, "Hydrated Magnesium Trisilicate in Peptic Ulceration," *Ibid.*, 1 (1936), 254.

(4) Mutch, "Magnesium Trisilicate," *Ibid.*, 2 (1937), 735.

(5) Levin, "Peptic Ulcer Therapy," *Am. J. Digestive Diseases Nutrition*, 4 (1937), 574.

(6) Levin, Burton, Roseman and Eisenberg, "Peptic Ulcer Therapy," *Rev. Gastroenterol.*, 6 (1939), 299.

(7) See review by Ross and Butler, "Magnesium Trisilicate—Clinical Observations of Its Medicinal Value and Chemical Methods of Testing," presented at the December, 1938, meeting of The American Pharmaceutical Manufacturers Association, at Washington, D. C.; also, Goldstein, "Antacid Gastric Therapy. The Use of Magnesium Trisilicate, Colloidal Kaolin and Aluminum Hydroxide," *Med. Record*, 148 (1938), 417, and Reid, "The Control of Gastric Hyperacidity by Magnesium Trisilicate," *Am. J. Digestive Diseases*, 6 (1939), 267.

(8) Gregory and Burr (edited by Friend), "A Text-book of Inorganic Chemistry," Charles Griffin and Co., Ltd., London (1926), Vol. III, Pt. II, p. 86.

(9) Döbereiner, "Analyse und Synthese des Meerschaums," *J. prakt. Chem.*, 17 (1839), 157.

(10) Lefort, "Mémoire sur les silicates," *J. pharm. chim.*, 39 (1861), 110.

(11) Von Ammon, "Ueber einige Silikate der Alkalien und Erden," Dissertation, Köln (1862) (summaries in Kopp-Will, "Jahresbericht über die Fortschritte der Chemie und verwandter Theile anderer Wissenschaften" (1862), p. 138, and Gmelin-Kraut, "Handbuch der anorganischen Chemie," 7th ed. (1912), Vol. III, Pt. I, p. 257).

(12) Heldt, "Studien über die Cemente," *J. prakt. Chem.*, 94 (1865), 157.

(13) Haushofer, "Mineralogische Mittheilungen," *Ibid.*, 99 (1866), 241.

(14) May, "Ueber einige künstliche Silikate," Dissertation, Breslau (1874), pp. 15, 21 (copies at The Library of Congress, Washington, D. C., and The Burton-Levin Foundation, Inc., Baltimore, Md.).

(15) Glass, "Notes on (the so-called) Magnesium Trisilicate," *Quart. J. Pharm. Pharmacol.*, 9 (1936), 445.

(16) Roseman, Levin and Eisenberg, "On the Nature of the Precipitates Obtained by Adding Sodium Silicate to Magnesium Sulfate," *Am. J. Pharm.*, 111 (1939), 400.

(17) Britton, "Electrometric Study of the Precipitation of Silicates," *J. Chem. Soc.* (1927), Pt. I, 425.

(18) Joffe, Kardos and Mattson, "The Laws of Soil Colloidal Behavior: XVII. Magnesium Sili-

cate—Its Base-exchange Properties," *Soil Sci.*, 40 (1935), 255.

(19) Kardos and Joffe, "The Preparation, Composition, and Chemical Behavior of the Complex Silicates of Magnesium, Calcium, Strontium and Barium," *Ibid.*, 45 (1938), 293.

(20) Schliephacke and Riemann, "Verfahren zur Abscheidung von Chlormagnesium aus Rohcarnallitlösungen und Abfalllaugen der Chlorkaliumfabrikation," Ger. Pat. 43,922 (1888).

(21) Kanter, "Sur l'acide silicique et les silicates alcalins et alcalino-terreux," *Bull. soc. chim.*, 29 (1903), 478.

(22) Jordis and Kanter, "Beiträge zur Kenntnis der Silikate. III. Zersetzung von Erdalkalisilikaten durch Wasser," *Z. anorg. Chem.*, 35 (1903), 336.

(23) Barth, "Verfahren zur Darstellung von Silikatfarben," Ger. Pat. 167,934 (1906).

(24) Jordis, "Beiträge zur Kenntnis der Metall-Silicate. I. Über die Darstellbarkeit von Metallsilicaten auf wässerigem Wege," *J. prakt. Chem.*, 77 (1908), 226.

(25) Jordis and Hennis, "Beiträge zur Kenntnis der Metall-Silicate. II. Über die Umsetzungen zwischen Natriumsilicat- und Metallsalzlösungen," *Ibid.*, 77 (1908), 238.

(26) Jordis and Lincke, "Beiträge zur Kenntnis der Metall-Silicate. III. Über die Umsetzung zwischen Natriumsilicat- und Eisenchloridlösungen," *Ibid.*, 81 (1910), 289.

(27) Pukall, "Fortschritte und fortschrittliche Bestrebungen auf dem Gebiete der Tonindustrie," *Ber.*, 43 (1910), 2095.

(28) Frohberg, "Ist mineralische Leimung von Druckpapier durch Magnesiumsulfat und Wasserglas möglich?," *Wochbl. Papierfabr.*, 44 (1913), 4250.

(29) Meding, "Verfahren zum Feuersichermachen von Gewebe, insbesondere zur Erzielung eines Flammenschutzes für Arbeitskleider der Sprengstoffindustrie," Ger. Pat. 337,842 (1921).

(30) Schwarz and Mathis, "Über Ammoniakate kieselsaurer Salze," *Z. anorg. allgem. Chem.*, 126 (1923), 55.

(31) Richardson, "The Action of Sodium Silicate When Used in Soaps. Part I—The Detergent Value of Sodium Silicate," *Ind. Eng. Chem.*, 15 (1923), 241.

(32) Igawa and Asahi Garasu Kabushiki Kaisha, "Improvements in or Relating to Manures," Brit. Pat. 218,401 (1924).

(33) Ishitani and Asahi Garasu Kabushiki Kaisha, "Improvements in Connection with Manures," Brit. Pat. 222,181 (1924).

(34) Ôta and Noda, "The Oxidase-like Actions of Colloidal Silicates," *J. Sci. Agr. Soc.* (Japan) No. 258, (1924), 287.

(35) Holmes and Anderson, "A New Type of Silica Gel," *Ind. Eng. Chem.*, 17 (1925), 280.

(36) Damiens, "Sur un silicate de magnésium artificiel," *Compt. rend.*, 180 (1925), 1843.

(37) Patrick, "Process of Producing Gels for Catalytic and Adsorbent Purposes," U. S. Pat. 1,577,186 (1926).

(38) Patrick, "Method of Preparing Gels for Catalytic and Adsorbent Purposes," U. S. Pat. 1,577,190 (1926).

(39) Ipatieff and Mouromtzeff, "La formation des silicates cristallisés en milieu aqueux sous pressions et à températures élevées," *Bull. soc. chim.*, 41 (1927), 1588; *Compt. rend.*, 185 (1927), 647.

(40) Grigorjew, "Über die Wechselwirkung zwischen Natrium-metasilicat und wasserlöslichen Metallsalzen," *Z. anorg. allgem. Chem.*, 167 (1927), 137.

(41) Grigorjew, "Die Theorie der Herstellung von Silica-gel mittels wasserlöslicher Metallsalze," *J. prakt. Chem.*, 118 (1928), 91.

(42) Vail, "The Culture of Certain Silicate Gardens," *Ind. Eng. Chem.*, 26 (1934), 113.

(43) Rembert, "Hydrous Silicate Gels and Method of Making the Same," U. S. Pat. 1,999,210 (1935).

(44) Kargin, Kats and Komovskiĭ, "Products of Reaction of Sodium Silicate with Electrolytes," *J. Applied Chem.* (U. S. S. R.), 10 (1937), 82.

(45) Smith, "Manufacture of Metal Silicates," U. S. Pat. 2,088,281 (1937).

(46) Mellor, "A Comprehensive Treatise on Inorganic and Theoretical Chemistry," Longmans, Green and Co., London (1925), Vol. VI, page 308.

(47) Clarke, "The Constitution of the Natural Silicates," *U. S. Geol. Survey*, Bull. 588 (1914), 14.

(48) Hildebrand, "Principles of Chemistry," 3rd Ed., The Macmillan Co., New York (1935), page 131.

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# A Study of Ephedrine with Silver Preparations*

*By D. J. McLeod and H. G. DeKay*†

Ephedrine, one of the alkaloids of Ma Huang, has been known and used in medicine for centuries. The plant from which the alkaloid is obtained is *Ephedra equisetina*, Bunge and *Ephedra sinica* commonly known as Ma Huang. The active principle, ephedrine, was isolated in 1885 by Yamanashi, in an impure form. Nagai, with the assistance of Hori (1), in 1887, isolated ephedrine in the pure form.

The chemical behavior of ephedrine has been exhaustively studied, and the litera-

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* An abstract of a thesis presented to the faculty of Purdue University in partial fulfilment of the requirement for the degree of Master of Science by D. J. McLeod, Eli Lilly & Co. Fellow.

† Associate Professor of Pharmacy, Purdue University.

ture is rich in references to derivatives and proof of chemical structure. Although the literature contains many references to ephedrine there are but a small number of discussions on the use of ephedrine with silver preparations.

The literature includes references to the preparation of many derivatives and homologues of ephedrine. Among the salts of ephedrine prepared are the hydrochloride, sulfate, oxalate, phosphate, nitrate, hydroiodide, hydrobromide, citrate, benzoate, salicylate and acetate. Quaternary ammonium derivatives have been prepared. A derivative recently reported is ephedrine camphorsulfonate.

Ephedrine hydrochloride and sulfate have commonly been dispensed by physicians in combination with silver proteinates for the bactericidal action of silver and the vasoconstrictor effect of ephedrine. The purpose of this investigation was concerned with the study of solutions of the ephedrine alkaloid and its salts with silver preparations, and with the preparation of silver salts of ephedrine.

Lanwermeyer (2) reports that ephedrine hydrochloride and ephedrine sulfate are incompatible with silver salts. Chen and Kao (3) state that chlorephedrine treated with silver nitrate in the presence of nitric acid yields pseudoephedrine. Solutions of the silver protein compounds precipitate alkaloids from their solutions. Waldbott (4) advances the theory that silver protein compounds, due to their alkalinity, precipitate alkaloids.

Further work on the incompatibility of alkaloids with certain silver proteins was carried out by Pilcher (5). He concludes from his experiments that the organic silver preparations inactivate epinephrine within a few hours. Therefore, epinephrine should not be used in these combinations unless freshly prepared.

Rebiere (6) reports that very dilute solutions of silver, gold or palladium salts, when heated with epinephrine in alkaline media, are reduced with the formation of a colloidal solution, and by the addition of a protective colloid the solution can be made stable.

Numerous compounds of silver with basic substances are described in the literature, but there is no reference to ephedrine silver compounds. In most of the basic compounds silver has a coördinate valency of two (7). The silver halide or nitrate is known to combine with two molecules of ammonia, pyridine, aniline, quinoline and like compounds.

The preparation of complex silver compounds of hexamethylenetetramine with silver chloride, silver oxalate and other silver salts has been reported. Complex salts of alpha amino acids, quinine silver phosphate, ethylenediamine silver phosphate and acriflavine have all been prepared and are recommended for use in place of silver nitrate. In this investigation, several complex silver salts of ephedrine were attempted.

## EXPERIMENTAL

*Preparation of Silver Salts.*—A number of silver salts were prepared and their reaction with ephedrine and its salts studied. Silver oleate, stearate and palmitate were prepared according to the procedure of Whitmore and Lauro (8). These salts were dry powders, insoluble in water, methyl alcohol, ether and chloroform. Oil emulsions of the silver soaps were attempted, but satisfactory preparations could not be obtained.

Complex silver salts of alpha amino acids were attempted using glycine and silver oxide (9). A stable complex salt was not obtained. The crystals produced decomposed before they could be separated from the reaction mixture and dried. The preparation of a complex salt, using silver sulfate and glycine, was attempted but failed.

Silver lactate was prepared by double decomposition of sodium lactate and silver nitrate. Grayish white, fluffy crystals, soluble in water, were obtained.

Hexamethylenetetramine silver nitrate was prepared by mixing dilute solutions of silver nitrate and hexamethylenetetramine (10). Fine, white crystals, unstable in aqueous solution, were obtained. A stable solution of hexamethylenetetramine silver nitrate was prepared using a large excess of hexamethylenetetramine.

Colloidal silver chloride was prepared by mixing a solution of silver nitrate with a sodium chloride, gelatin solution. Colloidal silver was prepared according to a method described by Schwyzer (11).

*Preparation of Salts of Ephedrine.*—Ephedrine stearate and oleate were obtained as liquids by neutralizing alcoholic solutions of the fatty acids with ephedrine. Ephedrine phosphate was prepared by neutralizing dilute phosphoric acid with the alkaloid. The tartrate was prepared by mixing a

Table I.—Reaction of One Per Cent Aqueous Solution of Ephedrine with Silver Salts

| Per Cent Solution of Silver Salt | Results |
|---|---|
| 1. Hexamethylenetetramine silver nitrate 0.5% solution | The silver salt was reduced to a silver mirror in two days |
| 2. Hexamethylenetetramine silver nitrate 0.5% solution with no ephedrine (control)* | The solution remained clear for ten days, then a silver mirror was produced |
| 3. Silver nitrate 5% solution | A brown precipitate was immediately produced. Upon standing the silver salt was reduced to a silver mirror |
| 4. Silver nitrate 5% (control) | Solution remained clear |
| 5. Hexamethylenetetramine solution | After eight days the silver salt was reduced to a silver mirror |
| 6. Hexamethylenetetramine solution (control) | Solution remained clear |
| 7. Silver lactate 5% solution | After three days the silver salt was reduced to a silver mirror |
| 8. Silver lactate 5% solution (control) | Remained clear |

* The control solutions contained no ephedrine.

methyl alcohol solution of ephedrine and tartaric acid and precipitating ephedrine tartrate with ether.

*Reaction of Ephedrine with Silver.*—In order that the reactions of ephedrine with different silver salts and preparations could be determined, a number of aqueous solutions of silver salts were added to one per cent aqueous solutions of ephedrine alkaloid. A one per cent solution of ephedrine with 5 per cent silver nitrate reduced the salt to metallic silver. Also a one per cent solution of ephedrine reduced silver sulfate in aqueous solution to metallic silver.

The action of ephedrine alkaloid was tested with the silver salts which were prepared in this work. The reaction of ephedrine with these silver salts is tabulated in Table I.

The silver soaps being insoluble in water were made into ointments containing one per cent of ephedrine alkaloid. Upon standing the ointments became very black and were characterized by a very disagreeable odor.

A series of experiments were completed using ephedrine salts with a saturated solution of silver salts and preparations to determine whether the alkaloidal salts had the reducing property of the base. One per cent aqueous solutions of ephedrine salts were mixed with a saturated solution of silver sulfate, a saturated solution of hexamethylenetetramine silver nitrate, a one per cent solution of silver lactate, and a 10 per cent solution of Neo-Silvol. In Table II will be found the results of the above experiments.

From the results of the experiments shown in Table II it is evident that the ephedrine salts do not have the reducing property of the free alkaloid.

A preparation of 5 per cent silver oleate in olive oil was made by incorporating 1.0 Gm. of ephedrine oleate thoroughly with the oil preparation. It darkened upon standing, but when compared with an oil preparation of silver oleate, free of alkaloid, they appeared to be the same. Apparently the ephedrine salt had no reducing action.

To solutions of colloidal silver chloride, one per cent ephedrine, one per cent ephedrine hydrochloride and one per cent sulfate were added. The results of these experiments are shown in Table III.

From the results obtained in the reactions of ephedrine with silver salts, the use of the free base, ephedrine, reduced the silver salts to metallic silver. However, if the basic property of ephedrine was neutralized by the formation of a salt and the salt used in solutions with silver salts there was apparently no reduction of the silver salts. Apparently, as shown in Table III, the use of ephedrine and ephedrine sulfate with colloidal silver was satisfactory.

Table II.—Effect of Ephedrine Salts in Solution of Organic Silver Preparations and Silver Sulfate

| | Ephedrine Solutions | Silver Solutions | Remarks | Cu-NaOH Test |
|---|---|---|---|---|
| *a.* | Ephedrine sulfate 1% | Silver sulfate sat. sol. | Solutions remained clear for three days, after that time a black precipitate formed | + |
| | Ephedrine sulfate control | | Clear solution | + |
| | | Silver sulfate control | Solution remained clear for three days | − |
| *b.* | Ephedrine sulfate 1% | Hexamethyleneamine $AgNO_3$ sat. sol. | Solution remained clear for seven days and then changed to a slight amber color. No precipitate | + |
| | | Control | After a few days, a slight deposit of silver | − |
| *c.* | Ephedrine sulfate 1% | Silver lactate 1% | Solution remained clear for 24 hours. No sediment. A fine black precipitate was formed | + |
| | Ephedrine lactate 1% | Silver lactate 1% | The same results obtained as the above solution | + |
| | | Silver lactate 1% (control) | Clear solution from one to twenty-five days. Fine black sediment was produced | − |
| *d.* | Ephedrine sulfate 1% | Neo-Silvol 10% | No change in appearance from that of the control | + |
| | | Neo-Silvol 10% (control) | The colloidal particles settle out on standing but were easy to disperse in solution by shaking | − |

Table III.—Reaction of Ephedrine and Ephedrine Salts with Colloidal Silver Chloride

| | Ephedrine Solutions | Colloidal Silver Chloride | Samples 1 and 2 | Cu-NaOH Test |
|---|---|---|---|---|
| 1. | Ephedrine 1% | One ounce | No change occurred in the solution after 14 days | + |
| 2. | Ephedrine hydrochloride 1% | One ounce | White precipitate immediately produced on standing | + |
| 3. | Ephedrine sulfate 1% | One ounce | No change occurred in the solution | + |
| 4. | ..... | One ounce (control) | Sedimentation occurs upon standing which is easily dispersed by shaking | − |

*Preparations of Ephedrine Silver Salts.*—Various compounds of silver with basic substances are described in the literature. It is known that silver halide or nitrate combines with two moles of ammonia, pyridine, aniline and other related substances. Ephedrine, being a basic substance, should combine with silver. The combination of a salt was attempted using freshly precipitated silver chloride and ephedrine in alcoholic solution. This mixture was refluxed for three hours. The silver salt was reduced to metallic silver. The process was repeated using ephedrine hydrochloride with the same result.

A preparation of a complex salt of ephedrine silver phosphate was undertaken. This compound was prepared by dissolving freshly precipitated silver phosphate in syrupy phosphoric acid, then adding ephedrine alkaloid until the solution was saturated. A yellow crystalline mass was obtained from the acid solution. This substance was partially soluble in water leaving an insoluble yellow residue. The residue was analyzed and found to be silver phosphate. The aqueous portion was evaporated nearly to dryness and colorless needle-shaped crystals were precipitated with alcohol. These crystals were identical with ephedrine phosphate.

The results of this experiment show that the acid solution was apparently a mixture of ephedrine phosphate and silver phosphate in phosphoric acid.

A preparation of colloidal silver with ephedrine was obtained that was apparently quite stable. This colloidal solution was made by adding a solution of silver nitrate to a gelatin ephedrine solution containing a small amount of 10 per cent ammonia water. A dark brown preparation was obtained which showed no tendency to form sediment. The colloidal solution contained 0.38 per cent silver.

Silver compounds of basic substances have been prepared by using the hydrochloride of the base. An example of this is silver arsphenamine dihydrochloride. An attempt was made to prepare silver ephedrine by using ephedrine hydrochloride and silver nitrate in methyl alcohol solution. This solution was refluxed for a period of three to four hours and then filtered. The filtrate obtained was evaporated to remove the methyl alcohol. A yellow crystalline residue was obtained which was soluble in water and methyl alcohol, but insoluble in ether. The crystalline residue was assayed for silver and found to contain 11 per cent silver. However, on recrystallizing the silver salt was precipitated and filtered off. The filtrate was evaporated to dryness and a compound was obtained that was free of silver. This crystalline substance was apparently an isomer of ephedrine. A silver compound with the basic substance, ephedrine, apparently could not be prepared.

It was shown in previous experiments that ephedrine was stable with silver in an acid solution. A double salt of silver ephedrine tartrate should be stable. A combination of ephedrine silver tartrate was prepared by mixing a methyl alcohol solution of ephedrine and tartaric acid with a methyl alcohol solution of silver nitrate. This reaction mixture was refluxed for several hours. A white precipitate was first formed which was silver tartrate. This precipitate, silver tartrate, was filtered off and the filtrate evaporated over a water-bath to remove the methyl alcohol. A syrupy amber-colored material was obtained which changed to a semi-crystalline substance on cooling and was soluble in water but insoluble in methyl alcohol and ether. The semi-crystalline mixture was thoroughly washed with ether; fine, slightly yellow powder was obtained which was soluble in water. An aqueous solution of the powder gave an acid reaction to litmus, a white precipitate was formed on addition of dilute hydrochloric acid, and a purple color was produced with the copper sulfate, sodium hydroxide reaction. The melting point of the dry powder ranges from 105–144° C. which indicates a mixture of two compounds and not a true chemical compound. This compound was found by analysis to contain 10.5% silver, and to be a mixture of ephedrine nitrate and silver tartrate.

## DISCUSSION

The results obtained in attempting to prepare silver compounds of ephedrine show that the basic property of ephedrine must be different from other basic organic compounds such as aniline, pyridine, quinoline and other like compounds. In the preparation of ephedrine silver chloride, metallic salt was apparently reduced to a silver oxide with no combination with the basic nitrogen of ephedrine. In the experiment preparing ephedrine silver nitrate, a mixture was obtained that upon recrystallizing from water precipitated a silver salt leaving a yellow crystalline compound free of silver. However, a colloidal solution of ephedrine and silver nitrate was prepared

and was apparently stable. The mixture of ephedrine silver tartrate obtained was apparently a stable mixture. The dry powder had not changed in appearance upon standing for one month.

## CONCLUSIONS

From the information obtained in this work the following conclusions may be drawn:

1. Ephedrine alkaloid in aqueous solution reduced silver salts to metallic silver. The ephedrine salts do not have the reducing property of the alkaloidal base.

2. Ephedrine or ephedrine sulfate appeared to be stable in solution with colloidal silver chloride. A colloidal solution of ephedrine silver nitrate was prepared which was apparently stable.

3. Ephedrine phosphate and silver phosphate, in strongly acid solution, were apparently stable. No chemical compound was produced. Ephedrine phosphate and silver phosphate were isolated and identified.

4. Silver compounds with ephedrine, such as ephedrine silver chloride and ephedrine silver nitrate, could not be prepared. Ephedrine hydrochloride was apparently converted into pseudoephedrine by the reaction of silver nitrate in alcoholic solution.

5. A mixture of ephedrine and silver tartrate was obtained that was apparently stable in aqueous solution. The compound was found by analysis to contain 10.5 per cent silver and to be a mixture of ephedrine nitrate and silver tartrate.

## BIBLIOGRAPHY

(1) Nagai, N., *Pharm. Ztg.*, 32 (1887), 700.

(2) Lanwermeyer, C. F., JOUR. A. PH. A., 20 (1931), 891.

(3) Chen, K. K., and Kao, C. H., *Ibid.*, 15 (1926), 625.

(4) Waldbott, S., *Pharm. J.*, 128 (1932), 282, 283.

(5) Pilcher, J. D., *J. Am. Med. Assoc.*, 88 (1927), 720.

(6) Rebiere, G., *C. A.*, 20 (1926), 477.

(7) Burrows, G. J., and Parker, R. H., *J. Am. Chem. Soc.*, 55 (1933), 3621.

(8) Whitmore, W. F., and Lauro, M., *Ind. Eng. Chem.*, 22 (1930), 646.

(9) Guggenheim, M., U. S. Pat. 1,417,167 (May 23, 1922).

(10) Grutzner, B., *Arch. Pharm.*, 236 (1898), 370.

(11) Schwyzer, J., *Quart. J. Pharm. and Pharmacol.*, 2 (1929), 116.

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# The Histology of Salvia Officinalis*

*By Elbert Voss† and Frank Fortunato*

The need for a thorough and comprehensive investigation of the histology of *Salvia officinalis*, L., became apparent when it was deemed of such importance as to merit a place in the National Formulary, Sixth Edition. An investigation of the literature revealed that in most cases *Salvia* was mentioned in connection with other species of the same genus, only in broad general discussions of the characteristic features of the *Labiates*, of which family it is a member. The number of different species belonging to this family is so large that many of them, including *Salvia*, have not individually received the meticulous histological study they deserve.

The early literature contains only brief and incomplete descriptions of *Salvia officinalis* leaf and stem, but there are many references to adulterants. The recent work by Youngken and Vander Wyk (1) on a sample of *Salvia* obtained from the Harvard University Garden, Cambridge, Massachusetts, does not entirely agree with the findings made in the course of the present investigation. Hence, it is obvious that to facilitate the exclusion of adulterants and to aid in the identification of genuine *Salvia*, a study of additional material obtained from another geographical source is needed. An amplification of the histology of this drug is, therefore, sufficient justification for a work of this kind.

The present monograph on *Salvia* in the National Formulary (2) gives a brief description of the leaf powder. It admits the presence of not more than ten per cent of the

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† Professor of Botany and Pharmacognosy, Duquesne University, Pittsburgh, Pa.

stems. The monograph, under "test for purity," makes only one brief specification in regard to *Salvia* stems; namely, that powdered *Salvia* shall not contain an abundance of reticulate tracheæ and crystal-bearing cells, both of which are present in *Salvia* stems; but no other reference is made to the histology of the stem.

In the present investigation equal attention has been given to the stem and the leaf.

The material used for study was obtained from the United States Department of Agriculture, Bureau of Plant Industry, Washington, D. C.

## HISTOLOGY OF THE LEAF

In cross sections of the leaf, regions common to the lamina and conducting areas of dorsiventral leaves are observed.

The cells of the upper epidermis of the lamina are quadrangular in outline, with thick, cutinized outer walls, and approximately every third or fourth cell gives rise to a trichome. The upper and lower epidermal cells of the midrib and veinal areas are smaller, with heavier and more wavy outer walls than those of the lamina. The cells of the lower epidermis are of very irregular sizes, being generally smaller and thinner-walled than those of the upper epidermis, with numerous larger ones giving rise to trichomes. Two rows of thick-walled hypodermal cells occur under the lower epidermis of the midrib. Passing from the upper epidermis toward the xylem of the midrib are four or five rows of collenchyma cells. The latter are followed by an area of relatively small parenchyma cells immediately above the xylem. From one to four rows of unmodified parenchyma cells occur at the sides of the fibrovascular bundle, and from three to five rows are found occupying the area between the phloem and the hypodermis of the lower surface.

The phloem area comprises almost half the fibrovascular bundle. The xylem area is composed of radially arranged and alternating rows of medullary rays and conducting cells. The latter are oval, round or polygonal in transverse outline, highly lignified and serve the function of support for the fibrovascular bundle. Medullary rays are one cell in width and usually alternate with single rows of conducting cells, but occasionally there may be from two to five rows of conducting cells filling the area between two medullary rays.

The lamina presents in general outline numerous undulations and thickened areas. The latter are due to a great increase in number of the spongy mesophyll parenchyma cells which project toward the upper epidermis. Both thickenings and undulations are accompanied by great variation in size and number of the mesophyll cells. Two rows of palisade cells, however, are most common. Occasionally there may be three rows at the crests of the undulations or near the margin, or occasionally one row at the crests of the thickened areas. In unmodified areas of the lamina there are usually four rows of spongy mesophyll cells.

## HISTOLOGY OF THE PETIOLE

The petiole in cross section is triangular in general outline, with rounded angles and concave surfaces

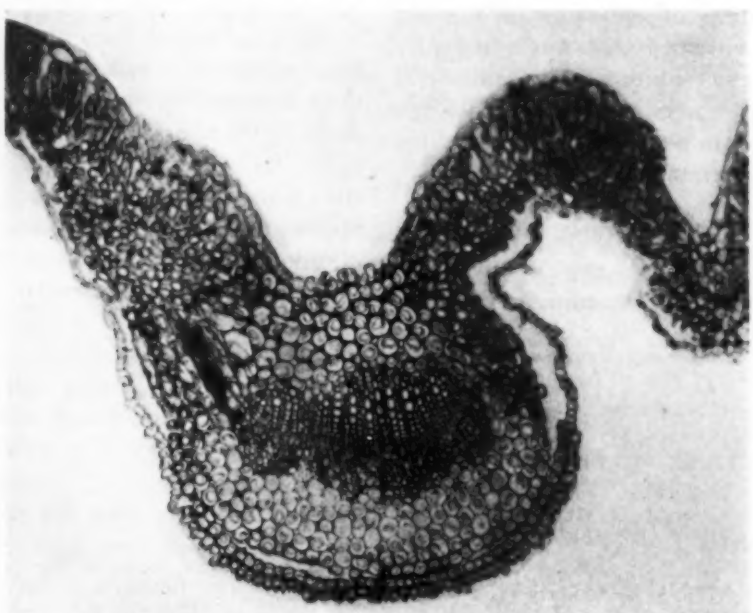

Fig. 1.—Photomicrograph. Transverse Section of Midrib. 100×.

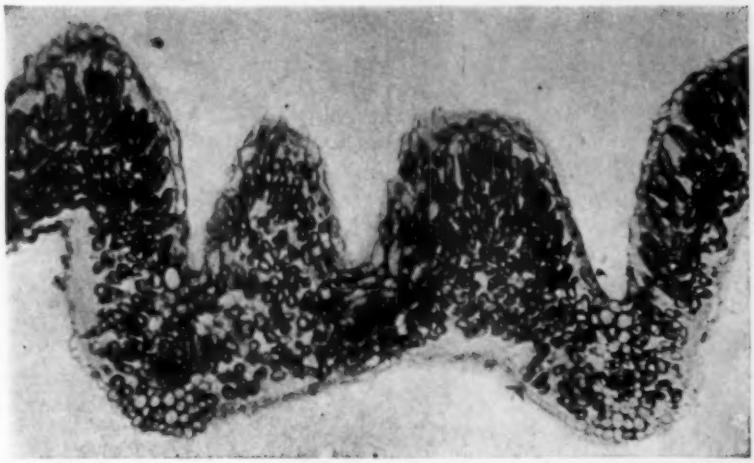

Fig. 2.—Photomicrograph. Transverse Section of Lamina. 100×.

between the angles. The epidermal cells are rectangular, with thick, cutinized and rounded outer walls, and numerous ones bear trichomes. Beneath the epidermis is found one to two layers of collenchyma cells, except in the angles where from four to six rows may occur. A large oval group of open collateral bundles at the center is surrounded by a broad cortex of thin-walled parenchyma cells. The smaller angles at the margins of the petiole each contain a small group of fibrovascular bundles similar to the large group in the center.

*Trichomes.*—Both glandular and non-glandular trichomes are numerous, with the non-glandular types present in greater number. The latter are uniseriate, from one to five cells in length, the individual cells distended or collapsed, and the end cell often attenuate and sometimes flexed. All trichomes are swollen at the base and usually seated on a short and broad pedestal. Three distinct forms of glandular trichomes occur: (*a*) one composed of a single-celled head with a thick cuticular wall supported by either a short and bulging stalk, or a straight, narrow, unicellular stalk; (*b*) a bicellular head supported by a bicellular stalk of varying length; (*c*) an eight-celled head with a very short, unicellular stalk.

*Isolated Tissues of the Leaf.*—The cells of the upper epidermis are devoid of stomata. They are polygonal in outline, with straight, heavy walls. Approximately every third cell bears a trichome, which is most commonly of the non-glandular type.

The lower epidermal cells are irregular or crescent-shaped in outline, with highly undulate margins. They contain numerous stomata which are surrounded by two epidermal cells lying transversely to the pore. There are proportionately fewer trichomes, with the glandular type relatively more numerous than on the upper epidermis.

The epidermal cells of the midrib and petiole are irregularly polygonal in outline with heavy straight walls. Trichomes, mostly non-glandular are present in great number, but no stomata are present.

The tracheal cells are greatly elongated, highly lignified and generally scalariform, occasionally spiral, in type.

Tracheids are highly lignified, usually spiral, but occasionally scalariform in type. The scalariform types are greatly elongated and very slender. The spiral types are of very irregular outline.

Parenchyma cells are elongated; some are very slender with thin unmodified walls, others distinctly rectangular with rather thick, pitted walls.

### HISTOLOGY OF THE STEM

Transverse sections of the stem, passing from periphery toward the center, present an epidermis, cortex, wood and pith. Very young stems show distinct open collateral vascular bundles separated by wide pith rays. Older stems show progressively deeper and broader vascular bundles, which, in mature stems, form a continuous woody cylinder composed of narrow, tangentially elongated, open collateral bundles separated by medullary rays usually one cell in width, but occasionally two cells in width. Young stems are distinctly quadrangular in shape, the older ones tend to become round but retain a greater thickness of the cortex in the areas corresponding to the angles of younger stems. A slightly quadrangular shape is, therefore, retained in mature stems.

The epidermal cells in transverse sections are of two types. Those between the angles are quadrangular, slightly tangentially elongated, the outer walls are thickened, and occasional ones give rise to trichomes. The epidermal cells covering the thickened areas at the angles are relatively smaller than those between the angles. They are practically isodiametric in outline, with heavy outer walls. Numerous ones bear trichomes.

Tangentially arranged groups of pericyclic fibers, located about one-third the distance from the cambium to the epidermis, divide the cortex into an inner and an outer region. Individual groups of these fibers vary from one to four cells in width, and from

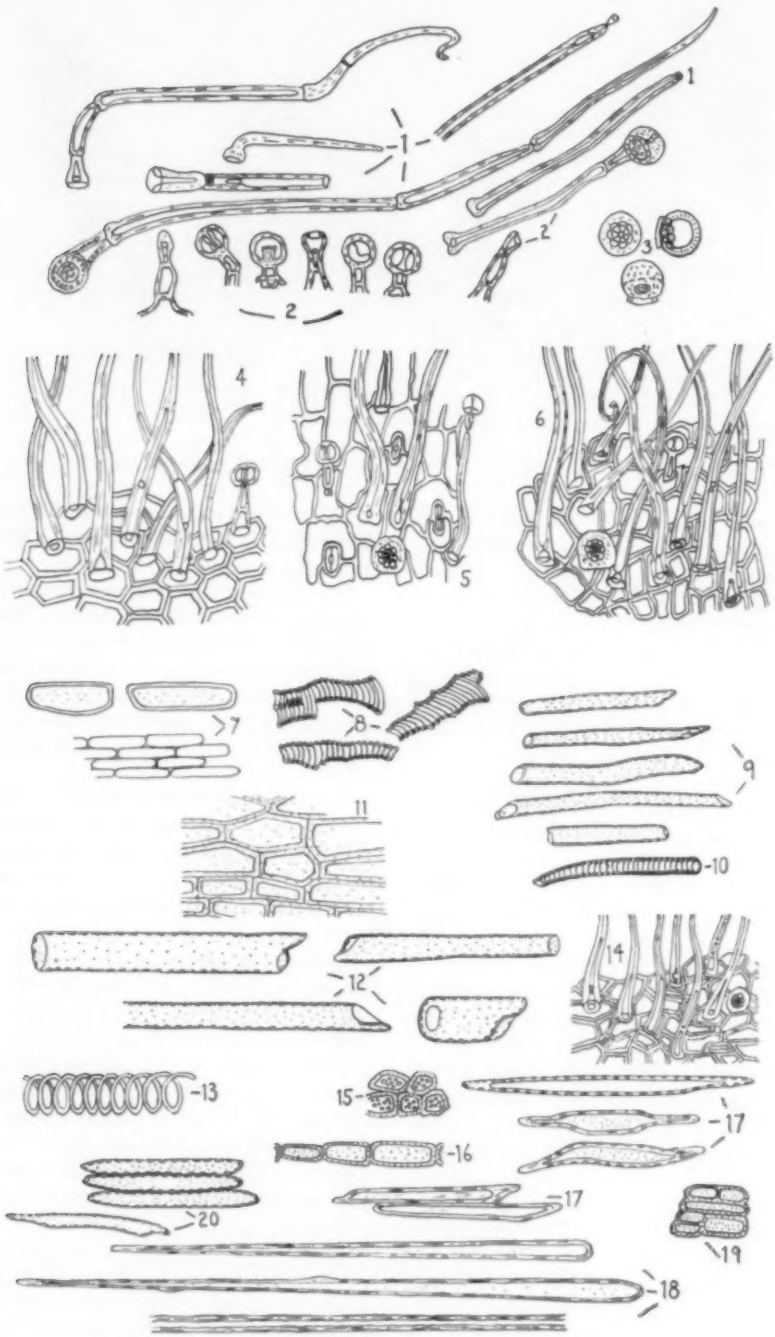


Fig. 3.—Isolated Tissues of Salvia officinalis.
(1) Non-glandular trichomes. (2 and 3) Glandular trichomes. (4) Upper epidermis of the lamina. (5) Lower epidermis of the lamina. (6) Epidermis of the petiole. (7 and 11) Parenchyma cells of the leaf. (8) Spiral and Scalariform tracheids of the leaf. (9) Scalariform trachael cells of the leaf. (10) Spiral tracheal cell of the stem. (12) Scalariform and reticulate tracheal cells of the stem. (13) Spiral tracheal cell of the stem. (14) Stem epidermis. (15) Crystal-bearing parenchyma cells of the stem. (16) Medullary ray cells of the stem. (17) Wood fibers of the stem. (18) Bast fibers of the stem. (19) Parenchyma cells of the stem. (20) Tracheids of the stem.

approximately three to fifteen cells, averaging approximately ten cells, per group. The larger groups are situated below the angles of the stem and smaller groups between the angles. In younger stems many of these fibers are unlignified, but in older stems all are heavily lignified. The cells of the outer cortex are tangentially elongated, thin-walled and irregular in outline. This area averages approximately seven rows of cells in width between the angles of the stem. Large groups of collenchyma displace the cells of the outer cortex at the angles of the stem, and account for the increase in thickness in these areas.

The woody cylinder is composed of xylem areas made up of vessels, tracheids and wood fibers, with parenchyma cells near the pith. The xylem areas are separated by medullary rays which are usually one cell, or occasionally two cells, in width. Medullary rays are more numerous below the angles of the stem, being rather infrequently found in areas midway between the angles. The latter areas also contain a relatively greater proportion of tracheids and wood fibers, with fewer vessels. All tissues of the xylem areas have generally a radial arrangement which is least apparent in areas about the larger vessels.

By far the most conspicuous elements of the xylem areas are the vessels which are polygonal in outline, with highly lignified walls, averaging approximately 0.025 mm. in diameter. They occur singly or in radially arranged groups of from two to six cells per group, and may be bordered by the medullary rays or by other xylem elements.

Tracheids occur interspersed among the smaller groups of vessels. They are relatively more numerous in the less vascular areas between the angles of the stem.

The most typical cells of the pith region are thin-walled, very large in diameter and isodiametric in outline. Numerous pith cells in the marginal areas are filled with a yellowish crystalline substance.

In radial sections the epidermal cells are slightly elongated and quadrangular in outline. The thin-walled parenchyma cells of the outer cortex are quadrangular and longitudinally elongated, while the more delicate cells of the inner cortex are of irregular outlines. Bast fibers are observed in some sections between the inner and outer cortex. Spiral and reticulate vessels with compactly arranged groups of tracheids and wood fibers between them, are apparent in radial sections, while medullary rays are very seldom seen. The first three or four outer rows of cells of the pith have fairly heavy walls, are elongated and polygonal in outline and occasional ones contain the yellowish crystalline substance observed in other sections. Passing toward the center of the stem the pith cells are less elongated and more isodiametric in outline.

In tangential sections the medullary rays vary from four to an indefinite number of cells in height and may even extend across the entire section, thus making it impossible to determine the actual number of cells present in some groups. Sections near the pith show numerous elongated parenchyma cells interspersed among vessels, tracheids and wood fibers.

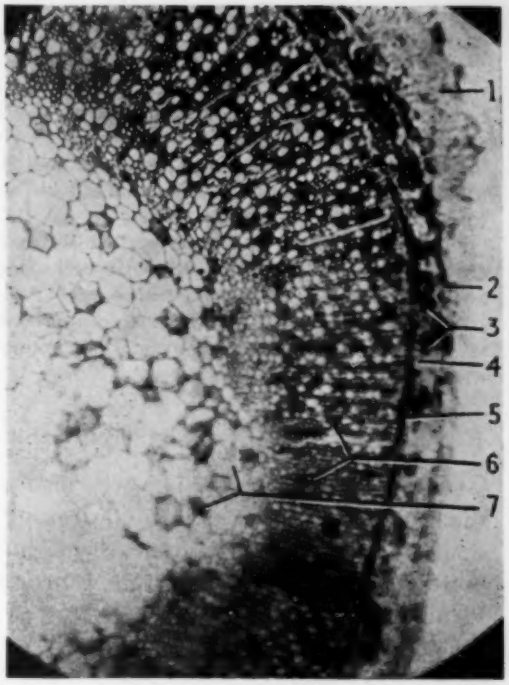


Fig. 4.—Photomicrograph. Transverse Section of Stem.

(1) Collenchyma. (2) Outer cortex. (3) Bast fibers. (4) Inner cortex. (5) Cambium. (6) Woody cylinder. (7) Pith.

*Isolated Tissues of the Stem.*—The stem epidermal cells like those of the petiole, are irregularly polygonal in outline, with heavy walls, and bear numerous non-glandular and occasional glandular trichomes. The trichomes are similar to those of the leaf.

The tracheal cells are both spiral and reticulate, varying from 0.100 mm. to 0.200 mm. in length and 0.015 mm. to 0.030 mm. in width. One end is usually round, the other diagonally extended. The side walls are heavily lignified and in reticulate types the markings frequently have a distinct scalariform arrangement. The spiral tracheal cells are comparable to the reticulate ones in size.

Tracheids are spiral or reticulate, and heavily lignified like the tracheal cells. They occur singly or in large groups when isolation has not been thorough. They are relatively much smaller than the vessels in transverse and longitudinal dimensions. They are greatly elongated in general outline with the ends either round or extended to an obtuse point.

The bast fibers approach or exceed the length of the longest non-glandular trichomes. Their side walls are heavy, and, in general, regularly thickened. A distinct cavity is usually present throughout the

length of the fiber. The ends are either round or acutely pointed.

Wood fibers vary in length, from one to three times the length of the tracheids. They are characteristically heavy-walled and highly lignified, the side walls are usually parallel with a broad cavity in the center. The ends may be round, but are usually extended to an acute or an obtuse point, and the latter may be straight or bent.

Pith cells bearing yellowish crystalline substance are conspicuous. Other pith cells may be unmodified, or possess irregular pit-like markings.

Medullary rays are distinguishable from the cells of the pith only by their longitudinal arrangement when seen in groups of two or more.

### SUMMARY AND CONCLUSIONS

A comprehensive study has been made of the histology of *Salvia* stem and leaf using a single sample obtained from the United States Department of Agriculture, Bureau of Plant Industry.

Our findings do not agree completely with those reported in the recent study made by Youngken and Vander Wyk on material from another source. The differences are significant enough to suggest that more than one variety of *Salvia officinalis* may now be in use as the official drug.

The present investigation reveals the following important histological characteristics of *Salvia*. These have not been emphasized in previous publications:

1. The occasional presence, in transverse sections of the stem, of medullary rays two cells in width.

2. Concentrically arranged groups of *bast* fibers dividing the cortex of the stem into inner and outer regions.

3. The relative abundance of medullary rays and conducting elements in xylem areas beneath the angles of the stem as compared to their occurrence between the angles.

4. Tangential sections of the stem revealing groups of medullary rays varying from four to an indefinite number of cells in height.

5. While no additional types of trichomes were noted on either stem or leaf, the absence of stalkless glandular trichomes with eight-celled heads is emphasized. The latter invariably possess short and broad, one-celled stalks. Glandular trichomes with one-celled heads have unicellular stalks. Those with two-celled heads show two-celled stalks, the latter varying greatly in length.

6. Leaves in cross section occasionally contain three rows of palisade cells at the crests of undulations or near the margin. Numerous thickened areas in the lamina are due to a great increase in the number of spongy mesophyll cells. These cells tend to displace, partially or entirely, the palisade mesophyll, and, together with the undulations of the lamina, give the latter an exceedingly irregular outline.

The above references to the variations in trichome and leaf structure should appear in the National Formulary monograph of *Salvia* leaf powder.

### REFERENCES

(1) Youngken, H. W., and Vander Wyk, R., "The Pharmacognosy of *Salvia officinalis*," *Bull. N. F. Committee*, VII (December, 1938) 3, 79–81.

(2) "The National Formulary, 1936 Edition," Mack Printing Co., Easton, Pa., pages 323–324.

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# A Study of Athyrium Filix-Foemina

*By Malcolm S. Trupp* and Forrest J. Goodrich†*

### INTRODUCTION

Many ferns are found growing natively in the State of Washington and several of them have been carefully investigated as to their chemical components. *Athyrium filix-fœmina* (Lady Fern) is a plant of unusual beauty and has attracted considerable attention. It is reported in the literature (1) as having anthelmintic principles in its roots, rhizomes and stipes. The purpose of the investigation was to determine whether or not it possessed anthelmintic principles similar to the official Aspidium and to ascertain the presence of significant components.

*Athyrium filix-fœmina* is a large non-evergreen fern found in the moist woods and near streams. The fronds are often more than a meter in length, pinnately two-three com-

* Instructor in Pharmacy, Saint Louis College of Pharmacy.

† Professor of Pharmacognosy, University of Washington.

pound; the venation of the pinnule is branched and margin is serrate. Sori are found on the lower surface of the older fronds. The underground stock is conical in shape, supported by many roots. Cross sections of the rhizome and stipe show some structures which differ from those of the official Aspidium. There is an absence of a lignified outer cortex or hypodermis. The xylocentric bundles are arranged in an interrupted circle and are five to six in number as compared to six to twelve for Aspidium.

## EXPERIMENTAL

The underground materials used in the investigation were collected from indigenous plants on the University of Washington campus. The product was air-dried and reduced to a No. 20 powder, and this material was used for all analyses except moisture determinations which were conducted on fresh samples. Duplicate determinations were made in all cases.

*Moisture.*—Ten-gram samples of the fresh material were dried to constant weight at a temperature of 100° C. Total moisture was 77.12 per cent. The moisture present in the air-dried material was 9.32 per cent.

*Selective Extraction.*—Ten-gram samples of the air-dried material were exhausted in a Soxhlet extractor for twenty hours with each of the following solvents and the solvents subsequently removed.

Table I.—Selective Extraction

| Solvent | Extractive at 105° C. in Per Cent |
|---|---|
| Petroleum ether | 0.68 |
| Ethyl ether | 0.37 |
| Chloroform | 0.25 |
| Alcohol (95 per cent) | 15.41 |
| Water | 11.99 |

*Ash.*—Total ash content was 5.22 per cent.

*Alkaloids.*—Twenty-gram samples were macerated in acid solution, alkalinized, filtered and the filtrate extracted with chloroform and ether. The evaporated extractives showed an absence of alkaloids when tested with various alkaloidal reagents.

*Glucosides.*—Various methods were tried in attempting to show the presence of glucosides.

First, The powdered material was exhausted with 70 per cent alcohol, the solvent removed under reduced pressure and the residue set aside for crystallization. The alcohol was kept neutral.

Second, The Rosenthaler process (2) was followed, using 20 Gm. of the drug, exhausting with 70 per cent alcohol for two hours, and the solvent kept neutralized with calcium carbonate. The alcohol was removed under reduced pressure and the residue taken up with water. The extraneous extractives were precipitated by lead acetate solution. The aqueous liquid was reduced to a syrupy liquid by vacuum distillation. This in turn was extracted with acetic ether. The material was extracted with water, clarified and shaken out with chloroform. The chloroform was removed by spontaneous evaporation.

None of the tests showed the presence of glucosides.

*Starch.*—The A. O. A. C. method was followed in the determination of starch. The yield was 3.67 per cent

*Pentosans.*—The A. O. A. C. method with phloroglucin and hydrochloric acid was followed. The amount present was 4.94 per cent.

*Sugars.*—The non-reducing sugars, calculated as sucrose and reducing, were determined by the A. O. A. C. official methods. The calculated sucrose was 3.42 per cent and the reducing sugars were 1.69 per cent.

*Tannin.*—The application of the usual qualitative tests for tannin on an aqueous extract of the drug indicated the presence of this substance. Five-gram samples of dried material were subjected to a quantitative determination as suggested in the official methods of the A. O. A. C. The tannin obtained amounted to 7.87 per cent of the samples taken for analysis.

*Filicic Acid.*—This organic material, being a physiologically active substance, it was of primary importance to learn whether it would be present in the rhizome of *Athyrium filix-fœmina* as in the official Aspidium. Although qualitative tests indicated the absence of this material, a determination was made on the ether extract using the official U. S. P. method for Oleoresin of Aspidium. This method, supplemented with that recommended by Kraft (3), gave negative results.

*Toxicity.*—White rats, fed the alcoholic and ether extracts of the rhizome in fairly large quantities failed to show any indications of debility or poisoning. This inability to demonstrate toxicity evidences an absence of a poisonous principle.

## SUMMARY

1. Differences in structure between *Athyrium filix-fœmina* and *Dryopteris Filix-mas* have been shown.

2. Although claims have been made for the toxicity and the presence of filicic acid in the rhizomes of *Athyrium filix-fœmina*, they seem unwarranted.

3. From the results obtained, it would seem safe to say that this fern rhizome or extracts of it would be unlikely to have any value as anthelmintics.

## BIBLIOGRAPHY

(1) Frye, "Ferns of the Northwest" (1934), page 124.

(2) Rosenthaler, "The Chemical Investigation of Plants" (1930), page 53.

(3) Kraft, "Foods and Drugs by Parry" (1911), page 469.

(4) United States Pharmacopœia XI (1936), pages 473–475.

(5) Pammel, "A Manual of Poisonous Plants" (1911), page 318.

(6) A. O. A. C., Third Edition, 27, (1930) 281–285; *Ibid.*, 18, (1930) 155–156.

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# Book Reviews

*A Textbook of Bacteriology*, by HANS ZINSSER and STANHOPE BAYNE-JONES. 8th ed., xxviii + 990 pages, 6¾ x 9⅝. New York: D. Appleton-Century Co., 1939. Price, $8.00.

This volume is the eighth edition of this well-known work on bacteriology. The volume, which is a comprehensive one, is divided into several sections covering the morphology and characteristics of bacteria, infection and immunity. More than 400 pages are devoted to the pathogenic micro-organisms. Diseases of uncertain origin and those caused by viruses are also discussed. Practical methods of bacteriology, immunology and serology are included. The section on protozoölogy carried in former editions has been omitted. The book is recommended to pharmacists who desire to have a comprehensive treatise of this character in their library.—A. G. D.

*Experimental Pharmacology and Materia Medica*, by DENNIS E. JACKSON, Ph.D., F.I.C.A., Professor of Pharmacology, Materia Medica and Therapeutics in the University of Cincinnati College of Medicine; formerly Associate Professor of Pharmacology, Washington University Medical School, St. Louis. 2nd ed., 906 pages, 892 illustrations, including 55 color plates. C. V. Mosby Company, 1939. Price, $10.00.

A systematically arranged series of experiments in pharmacodynamics selected from the author's experience in teaching the subject is presented in this volume, which is profusely illustrated. The technique of all operations is described in detail, which is believed to be an advantage in books of this type. Although the book is written for medical students, there is sufficient material in it of pharmaceutical application to make it a valuable reference work for students in pharmacy. Parts of the book deal with materia medica and presciption writing and incompatibilities. There are also a solubility table, Latin-English vocabulary, a list of abbreviations and an extensive list of supply and equipment houses. The book should be in every pharmacy college library.—A. G. D.

*The Chemistry of Organic Compounds*, by JAMES BRYANT CONANT, rev. by MAX TISHLER. x + 658 pages, 5½ x 8⅜. New York: Macmillan, 1939. Price, $4.00.

This edition of the book has been revised by Max Tishler of Merck and Company. The order of presentation is the same as that in the first edition and in general conforms to the plan commonly used. Some space is given to the discussion of vitamins and hormones and there is a short chapter on drugs. A chapter is devoted to the amino acids and another to the proteins. The formation of chelate rings and the structure of the guanidinium ion are some of the new subjects discussed. The volume has proved its value as a text for general courses in organic chemistry and is recommended to pharmacists who desire to become acquainted with the newer theories in organic chemistry.—A. G. D.

*Quantitative Biological Spectrometry*, by ELMER S. MILLER. iii + 213 pages, 8¼ x 10¾, "Mimeoprinted." Minneapolis: Burgess Publ. Co., 1939. Price, $3.50.

This laboratory manual has been developed for the course in applied spectroscopy at the University of Minnesota. A description is given of the instruments used in spectroscopy, their principles of operation and of methods used. Various applications of spectroscopic methods are described, such as: the demonstration of chemical constitution, identification of hemoglobin and other pigments, vitamins, etc.; determination of lipids. The chapter on vitamins should be of special interest to pharmacists.—A. G. D.

*Standard Chemical and Technical Dictionary*, by H. BENNETT. xlii + 638 pages, 6 x 9. New York: Chemical Publishing Company, Inc., 1939. Price, $10.00.

This dictionary is stated to contain 25,000 definitions in addition to organic names, prefixes, symbols, etc. The definitions are concise, perhaps too concise in some instances, and some of the new drugs such as sulfanilamide and sulfapyridine are not included. The apothecaries' symbols, which are given in the appendix, are incorrect in part and give evidence of having been carelessly assembled. In spite of these deficiencies, the dictionary should be of value to pharmacists as a source of information.—A. G. D.